EFFECTS OF CAPTOPRIL ON THE HEART

mechanisms and therapeutic potentials

Pieter A. de Graeff

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STELLINGEN

1. De behandeling van congestief hartfalen met een angiotensine converting enzym remmer, eventueel in combinatie met een diureticum, dient in een vroeg stadium aangevangen te worden.

2. Het is te verwachten dat het geven van captopril aan patienten na het myocardinfact tot een afname van de cardiovasculaire morbiditeit en mortaliteit zal leiden.

3. Door de aanwezigheid van de sulfhydryl groep in het molecuul van captopril kan de stof het effect van de behandeling met nitraten versterken en het optreden van tolerantie tegengaan.

4. In het hart is een plaatselijk kallikreïne-bradykinine systeem aanwezig dat als tegenhanger fungeert van het cardiale renine-angiotensine systeem.

5. Anti-aritmische eigenschappen van angiotensine converting enzym remmers bij patienten met matig tot ernstig hartfalen berusten hoofdzakelijk op het kalium-retinerende effect van deze middelen.

6. All angiotensin converting enzyme inhibitors are equal, but some angiotensin converting enzyme inhibitors are more equal than others.

7. Het hart is een endocrien orgaan dat een centrale rol speelt bij de regulatie van de water- en zouthuishouding.

8. Bij de nog onverklaarde fenomenen stille ischaemie en syndroom X speelt een verminderde activiteit van bradykinine vermoedelijk een belangrijke rol.

9. Selectieve IgA deficientie is niet zo selectief als aanvankelijk aangenomen werd.

10. De specifieke, lokale acties van geneesmiddelen in verschillende organen vragen om meer accent op het gebied van "drugtargeting".

11. Voor een objectieve beoordeling van geneesmiddelen ter registratie is het dringend gewenst dat negatieve resultaten van klinisch-farmacologisch onderzoek vaker gepubliceerd worden.

12. Het College ter beoordeling van geneesmiddelen is in Nederland een bij de wet geregeld zelfstandig orgaan. Deze constructie verdient de voorkeur boven de situatie in de meeste landen waar sprake is van een adviesorgaan van de overheid.

13. Meldingen over de frequentie van optreden van afwijkingen waarbij de parameters moeilijk nauwkeurig zijn vast te stellen geven doorgaans een percentage van 20%.

14. Zorgen voor het milieu is veel belangrijker dan zorgen over het milieu.

15. Promoveren in mei heeft iets natuurlijks.

Stellingen behorende bij het proefschrift van P. A. de Graeff
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aan mijn ouders

voor Geeske

It is much easier to write
upon a disease
than upon a remedy.

The former is in the hands of Nature
and a faithful observer with
an eye of tolerable judgement
cannot fail to delineate a likeness.

The latter will ever be subject
to the whim,
the inaccuracies and
the blunder
of mankind.

William Withering 1741-1799

CONTENTS

CHAPTER I

GENERAL INTRODUCTION AND AIMS OF THE THESIS

"Thou, gentle viper", with these words Alberto Zanchetti began his introduction to a symposium in 1982 on the developing therapeutic concept of angiotensin converting enzyme inhibition (Zanchetti, 1982). The viper he referred to, was the poisonous Brazilian snake, Bothrops jararaca. In 1968, a mixture of peptides was extracted from its venom which was able to inhibit the conversion of angiotensin I into angiotensin II (Bakhle, 1968). Three years earlier this crude extract of peptides had already been shown to have a strong bradykinin potentiating activity and was named bradykinin potentiating factor or BPF (Ferreira, 1965). BPF inhibited plasma kininase II activity and potentiated the spasmogenic activity of bradykinin upon isolated smooth muscle preparations. Subsequently, nine low molecular weight pharmacologically active peptides were isolated, demonstrating both kininase inhibition and inhibition of enzymatic angiotensin I conversion (Ferreira et al., 1970).

It was at this stage that Cushman and Ondetti carried out their pioneering work of synthesizing specific inhibitors of the angiotensin converting enzyme (Ondetti et al., 1971). This resulted first in the development of a nonapeptide, teprotide, and opened the way for the birth and development of the concept that angiotensin converting enzyme inhibition can be successfully employed in the therapy of cardiovascular disorders, such as arterial hypertension and congestive heart failure. Preliminary clinical studies demonstrated the great potential of teprotide as a novel antihypertensive and vasodilating agent, limited only by its lack of oral activity (Gavras et al., 1974; Curtiss et al., 1978). The therapeutic efficacy of teprotide provided the last evidence that an orally active antagonist of the angiotensin converting enzyme was highly advantageous in clinical use. In 1976, the same team that had developed teprotide synthesized the first orally active angiotensin converting enzyme inhibitor, captopril (Ondetti et al., 1977).

The pharmacological properties of captopril were first evaluated in 14 normotensive volunteers in 1976 (Ferguson et al., 1977). This was followed by a great number of trials in patients with hypertension and heart failure. Already in 1979, it was concluded that captopril was a welcome addition to the therapeutic repertoire (Atkinson and Robertson, 1979). Due to severe side-effects such as skin rash, neutropenia and nephrotic syndrome, its use was initially restricted to patients with otherwise uncontrollable disease. By limiting the use of captopril to high doses in complicated cases, especially those with renal failure and/or collagen vascular disease, a very misleading picture of toxicity

emerged. Subsequently, it was discovered that many of these side effects were dose related and could be avoided by using less than 150 mg/day with little or no loss of efficacy (Heel et al., 1980). Due to this increased benefit/risk ratio, its reputation increased and in 1982, as written by Zanchetti in his introduction, a place for captopril in the treatment of hypertension and congestive heart failure was definitely established (Zanchetti, 1982).

This coincided with the increasing popularity of vasodilator therapy as an important adjunct in the management of severe heart failure (Braunwald, 1977; Cohn, 1977). Therapy for cardiac failure had traditionally involved the administration of an inotropic drug to increase the contractile force of the heart and a diuretic to augment the renal excretion of salt and water. While often effective in the treatment of mild congestive heart failure, this approach did not consider the deleterious effects that peripheral vasoconstriction plays in further compromising the heart (Levine, 1985).

This can be explained as follows. When pump function deteriorates progressively and cardiac output decreases, a number of neurohumoral mechanisms, especially the sympathetic nervous system and the renin- angiotensin system, are activated in order to maintain blood pressure and, to a lesser extent, cardiac output. Arteriolar vasoconstriction results in an increased vascular resistance, and venoconstriction causes an increase in both venous return and left ventricular filling pressure. The normal heart, with an adequate reserve, can easily respond to the increase in aortic impedance by maintaining a normal cardiac output, or it can respond to an increase in preload by generating more systolic contractility and increasing stroke volume. Because the failing heart lies on a much flatter Frank-Starling curve, increases in preload and afterload add to the workload of the heart and further decrease pump function, which in turn activates more neurohumoral vasoconstriction, and thus a vicious circle is established. Ventricular dysfunction is exacerbated by neurohumoral vasoconstrictor mechanisms and vice versa (Francis, 1985; Parmley, 1985; Levine, 1985). The rationale of vasodilator therapy is to interrupt this vicious circle by improving the working environment of the heart.

This theoretical appeal of using vasodilators to treat heart failure was subsequently reinforced by numerous studies demonstrating salutary hemodynamic and clinical effects in patients with left ventricular failure, acute and chronic, secondary to a variety of causes (Braunwald, 1977). Several vasodilators were tested, varying in their hemodynamic effects, site and duration of action, and mode of administration (Abrams, 1985; Franciosa et al., 1984). The earlier studies involved long-acting nitrates, which, being predominantly venodilators, markedly lowered ventricular filling pressure while less strikingly raising cardiac output. Direct acting primary arterial dilators, such as hydralazine and minoxidil, were subsequently shown to raise cardiac output significantly, while slightly reducing ventricular filling pressures. Regimens with balanced

arterial and venodilating properties combined these two effects. Thus, nitrates in combination with hydralazine or prazosine alone were thought to be useful. Nitroprusside appeared to have the most ideal hemodynamic profile, but unfortunately this drug could only be given intravenously (Franciosa and Cohn, 1978).

Captopril appeared to have many advantages over these older agents (Braunwald and Colucci, 1984). It exerted a balanced action on the arterial and venous blood vessel without baroreceptor reflex tachycardia. It produced long-term hemodynamic benefits in contrast to some other vasodilators, especially the αl-receptor antagonist prazosine. Exercise tolerance time and the New York Heart Association functional class improved significantly when compared to placebo. This led to the conclusion that captopril was an effective oral agent for the management of heart failure resistant to optimal digitalis and diuretic therapy. However, it was not yet clear whether it should be considered a "first-line" agent before instituting therapy with other vasodilators (Romankiewicz et al., 1983).

It was inevitable that the success of captopril should instigate the development of numerous new compounds inhibiting the angiotensin converting enzyme (Brunner et al., 1985). The main differences between most of these new angiotensin converting enzyme inhibitors and captopril was a longer duration of action and the absence of a sulfhydryl-group. It was assumed that this would lead to a less frequent dosing schedule on the one hand and a reduction of side-effects on the other. The molecules of captopril and of three well- known non-sulfhydryl containing angiotensin converting enzyme inhibitors are depicted in Figure 1. The first representative of this new class of angiotensin converting enzyme inhibitors was enalapril. Initial results in patients with heart failure demonstrated similar hemodynamic effects compared with captopril, with a slower onset and a longer duration of action (Turini et al., 1983). Enalapril was

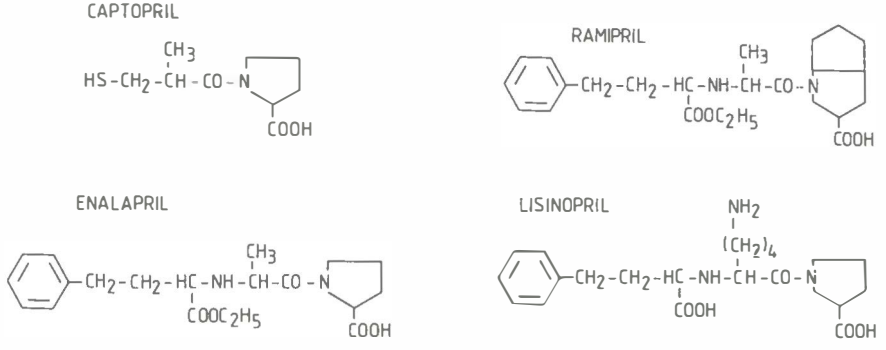

Figure 1. The molecules of captopril, enalapril, ramipril, and lisinopril.

quickly succeeded by two other long-acting angiotensin converting enzyme inhibitors, namely lisinopril and ramipril, both capable of complete inhibition of the angiotensin converting enzyme (Biollaz et al., 1981; Witte et al., 1984). However, little was known about the comparative efficacy and safety of such long-acting angiotensin-converting enzyme inhibitors in patients with heart failure.

It was at this point that our studies with captopril in heart disease were started. Two questions were raised. The first involved the comparison between short- and long-acting angiotensin converting enzyme inhibition in heart failure. For this purpose, we studied the acute and chronic effects of captopril and ramipril in patients with moderate to severe congestive heart failure, with special attention being focused on renal effects and cardiac arrhythmias.

The second question addressed a fundamental issue. Did captopril have a direct effect on the heart, independent of the pre- and afterload reducing properties? This question was based on the evidence that renin-like substances are ubiquitous enzymes which can probably be found in most tissues, especially the heart (Ganten et al., 1976). This would mean that the heart is capable of locally synthesizing angiotensin II, which could play an important role in certain pathological conditions. Furthermore, other mechanisms of action of captopril besides inhibition of angiotensin II were known to be present, especially the aforementioned potentiation of bradykinin. Although no rise in plasma concentration of bradykinin had been described, this did not exclude the possible important accumulation of kinins in the tissues (Johnston et al., 1982). Little was known about the effect of bradykinin on the heart. Finally, it had been described that captopril interferes with adrenergic transmission both via pre- and postjunctional actions (Antonaccio et al., 1981; Hatton et al., 1982). Both angiotensin-dependent and, at higher doses, angiotensin-independent actions are involved (Saruta et al., 1982; Collis et al., 1981).

The problem in studying these direct effects of captopril in patients is that the (indirect) effects on pre- and afterload are so pronounced, that any direct effect on the heart itself may be masked. Therefore, we decided to test the effect of captopril in the isolated rat heart, perfused as described by Langendorff. In order to activate possible mechanisms, acute ischemia and subsequent reperfusion were produced by reversible ligation of the left coronary artery. Pronounced effects of captopril were found to be present, which appeared to be beneficial to the heart (Van Gilst et al, 1984). Captopril reduced ventricular fibrillation and the loss of high energy phosphate nucleotides and thereby partly maintained mechanical function impaired by ischemia and reperfusion. This was the starting point for a number of studies, all addressing the cardioprotective properties of captopril. In order to study these effects in vivo, we developed a closed-chest pig model in which reversible occlusion of the left coronary artery was achieved with a balloon catheter. Eventually we proceeded

to the clinical situation. The effects of captopril were studied in patients undergoing thrombolysis after acute myocardial infarction, as this closely mimicks the situation of ischemia and reperfusion.

During the isolated rat heart studies, a vasodilating effect of captopril on the coronary blood flow was found, which appeared to be partly related to its sulfhydryl group. Since it was known that other sulfhydryl containing compounds, such as acetylcysteine, potentiate the hemodynamic response to nitrates (Torresi et al., 1985; Packer et al, 1987c), this interaction was explored further, both in the experimental and clinical situation.

The present thesis summarizes our studies on the differential effects of captopril in the heart and its possible role in certain pathophysiological conditions of heart disease. This work was supported by findings of other groups. During recent years more knowledge has accumulated concerning the relationship between the renin-angiotensin system and heart structure and function and of the influence of captopril and other converting-enzyme inhibitors, both in the experimental and clinical situation. Perhaps this is best exemplified by an article in 1988 from the same Alberto Zanchetti, who paid such a generous tribute to the Bothrops Jararaca in the past. In another introduction to a symposium on the renin-angiotensin system, he wrote about the heart as the location, target and regulator of the renin-angiotensin system. Furthermore, he speculated on the cardioprotective effects of angiotensin converting enzyme inhibitors in the management of cardiac ischemia, including acute myocardial infarction and the subsequent recovery period (Zanchetti, 1988).

In the following chapters the conclusions of our results will be placed in a more general context. Three topics are under discussion. The first relates to the mechanisms of action which may be involved in the different effects of captopril and other converting-enzyme inhibitors in heart disease (chapter II). Special attention will be given to the contributory role of the sulfhydryl group in the pharmacological effects of captopril. Next, our animal experiments will be compared with those of other investigators who have studied similar and differential cardioprotective effects of angiotensin converting enzyme inhibitors (chapter III). Finally, the clinical relevance and possible new indications of captopril will be discussed in chapter IV. Following the conclusion and final remarks (chapter V), the main studies, which have been published or are submitted for publication, are added as appendices. These include data on:

a. The acute and chronic effects of captopril, in comparison with ramipril, in patients with moderate to severe congestive heart failure (nos. 1 and 2).

b. The effect of captopril on myocardial damage and ventricular arrhythmias during ischemia and reperfusion in the isolated rat heart (nos. 3, 4 and 6), in the closed-chest pig model (nos. 5 and 6) and in patients undergoing thrombolysis after acute myocardial infarction (no. 7).

c. The effects of captopril on coronary blood flow and the interaction with nitrates in the isolated rat heart (no. 8) and in patients with ischemic heart disease and angina pectoris (no. 9).

CHAPTER II

MECHANISMS OF ACTION

1. RENIN-ANGIOTENSIN SYSTEM

Circulating vs. tissue renin-angiotensin system

The renin-angiotensin system is traditionally thought of as a homeostatic feedback loop in which the juxta-glomerular cells of the kidney, in response to a variety of stimuli, of which the most important are hypotension or diminished delivery of sodium to the distal tubular macula densa sites, secrete the enzyme renin. Renin in turn circulates in blood and cleaves a hepatically synthesized α2-globulin (angiotensinogen), thereby generating the decapeptide angiotensin I. The latter is subsequently converted by the angiotensin-converting enzyme (or kininase II) to the octapeptide angiotensin II.

This is the physiologically active component of the renin-angiotensin system. It acts throughout the body to produce a number of cardiovascular, metabolic, and behavorial effects (Oparil and Haber, 1974; Reid, 1985). The main actions are depicted in Figure 2. Most importantly, angiotensin II is an extremely potent pressor agent. It stimulates the secretion of aldosterone by the adrenal gland and feeds back directly on the juxtaglomerular cells to at least partially suppress renin secretion. Effects on the sympathetic nervous system

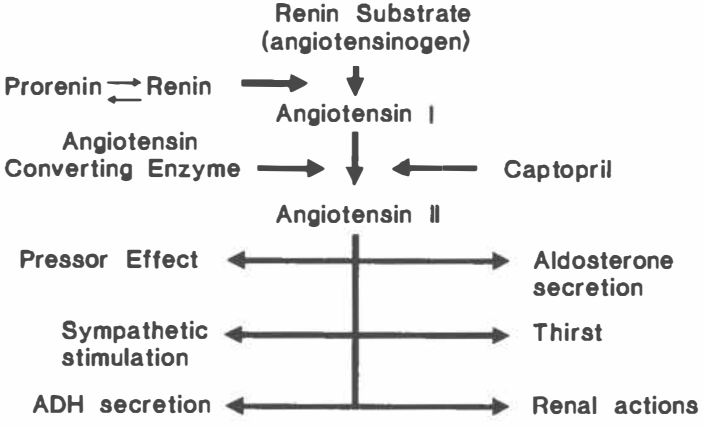

Figure 2. The renin-angiotensin system and the main actions of angiotensin II. Captopril inhibits the angiotensin converting enzyme.

are discussed later in this chapter. Renal actions include inhibition of the secretion of renin (feed-back mechanism), reduction in renal blood flow, especially of the efferent arteriole, and increase in proximal tubular sodium reabsorption. Angiotensin II also acts on the brain and pituitary to stimulate drinking and to increase the secretion of vasopressin (ADH). However, the clinical relevance of these central effects remains to be established.

The renin-angiotensin system was initially regarded as a system present in the circulation and dependent on the effects of renin and angiotensin production in plasma. Angiotensin II did not seem to to play an important role in the healthy animal or human subject in sodium balance. However, it became crucial in the maintenance of blood pressure after sodium depletion (Oparil and Haber, 1974; Haber, 1976). This was corroborated by elevated plasma renin activity in patients during sodium depletion, anesthesia, and haemorrhage, as well as in the initiation of renovascular hypertension and in the development of congestive heart failure (Reid, 1985). However, during the chronic phase of these conditions, plasma renin activity was frequently normal (Dzau, 1987a). Therefore, its primary function appeared to be a short-term cardiorenal homeostasis system.

During the past 15 years, this classical concept of the circulatory renin-angiotensin system has radically changed, due largely to the development of multiple inhibitors of the system (especially the angiotensin converting enzyme inhibitors) and the advent of sensitive radioimmunoassay methods for determination of virtually every component of the system. It became increasingly clear that angiotensin II is not only generated in plasma but also in other tissues, including the heart. These tissue renin-angiotensin systems operate in whole, or in part, independently of the circulatory renin-angiotensin system and may play a crucial role in the pathophysiology and symptomatology of heart disease (Campbell, 1987; Dzau, 1988a; Dzau, 1988b; Re 1987a; Lindpainter et al., 1987).

Evidence for the existence of vascular and cardiac renin-angiotensin systems

Although renin-like enzymes had been extracted from a variety of organs other than the kidney, including the uterus, placenta, heart, brain, adrenal glands and the submaxillary salivary gland (Ganten et al., 1976), it was initially thought that these extrarenal sources did not play any physiological role in cardiovascular homeostasis. However, this idea started to change when clinical studies with angiotensin converting enzyme inhibitors demonstrated that although the acute blood pressure lowering vasodilating effect of angiotensin converting enzyme inhibitors correlated with the initial plasma renin activity, the chronic response appeared to bear little relationship to pretreatment plasma hormonal levels (Dzau, 1986). This was further supported by studies

that demonstrated that chronic administration of angiotensin converting enzyme inhibitors also lowered blood pressure when plasma renin activity was not elevated (Gavras et al., 1978) or even low, as in anephric subjects (Man in 't Veld et al., 1980).

Direct evidence for an inhibition of angiotensin converting enzyme in various tissues, including the aorta, was subsequently provided in spontaneously hypertensive rats (Cohen and Kurz., 1982; Unger et al., 1984; Unger et al., 1985). Furthermore, the prolonged antihypertensive action of the converting enzyme inhibitors was unrelated to angiotensin converting enzyme inhibition in the plasma but was associated with a persistent tissue angiotensin converting enzyme inhibition in the kidney and vascular wall. This led to the hypothesis that the vascular wall renin-angiotensin pathway plays an important role in cardiovascular homeostasis (Dzau, 1986). Further studies confirm this hypothesis. Using sensitive techniques both renin messenger RNA and angiotensinogen messenger RNA were detected in the vascular wall, demonstrating beyond doubt that angiotensin II can be generated locally in the vascular wall. (Dzau, 1987b). The latter led to the revised concept that the primary function of the circulating renin-angiotensin system is not systemic delivery of angiotensin II to the tissues, but rather the delivery of angiotensinogen and prorenin (Campbell, 1987). Angiotensin I and II are generated largely locally within the tissues by the action of plasma-derived renin on plasma-derived angiotensinogen and the action of tissue angiotensin converting enzyme. It is suggested that this plasma-derived renin represents prorenin, the inactive form of renin which is activated by mechanisms in the vascular wall (Dzau, 1987b). This renin-angiotensin system may play a major role in the pathophysiology of hypertension (Dzau, 1986).

Now what about the heart? As early as 1973 it was demonstrated in the blood-perfused Langendorff heart that angiotensin I could be converted into angiotensin II when administered into the coronary artery (Gerlings et al., 1973). Several other investigators subsequently confirmed this cardiac generation of angiotensin II from angiotensin I in guinea pig atria (Ziogas et al., 1984), in the coronary vasculature of the rabbit (Needleman et al., 1975) and in the isolated perfused rat heart (Xiang et al., 1985). A requirement for the demonstration of local synthesis of proteins and peptides is the expression of their genes in the tissues. This was obtained by biochemical characterisation of renin-angiotensin components in the heart and the demonstration of renin and angiotensinogen messenger RNA's in the heart, proving that the heart itself is capable of synthesizing angiotensin II (Lindpainter et al, 1988; Dzau, 1988a)

The physiological role of the cardiovascular renin-angiotensin system

The physiological role of locally generated angiotensin II may be manifold (Dzau, 1988a-d; Re, 1987a,b). Angiotensin II causes intense coronary vasoconstriction. It may also directly influence cardiac contractility, as high concentrations of myocardial receptors for angiotensin II have been demonstrated in various animal species. Another speculative function of cardiac angiotensin II is the development of cardiac hypertrophy either by a direct intracellular effect or mediated via activation of the cardiac sympathetic neurotransmission. This local enhancement of the neurotransmitter noradrenaline may also have chronotropic and inotropic consequences, as will be discussed below. Finally, local angiotensin II may interact with the atrial natriuretic peptide (ANP) which antagonizes the physiological effects of angiotensin II (Laragh, 1985).

ANP appears to be a defense mechanism against detrimental effects of angiotensin II on the heart. In isolated working rat hearts it has been shown that ANP protects against the sequels of ischemia and reperfusion to which a functional antagonism between ANP and angiotensin II may contribute (Schölkens et al., 1988a). Following myocardial infarction a gradual and marked rise in ANP has been described both in rats (Hodsman et al., 1988a; Michel et al., 1988) and in man (Tomoda, 1988), correlating with hemodynamic changes and the development of cardiac hypertrophy. Salt and water retention may be prevented or limited either directly or indirectly, by inhibiting the renin-angiotensin system (Hodsman et al., 1988a). Angiotensin converting enzyme inhibition can reverse this increase (Michel et al., 1988). A resetting of ANP plasma levels has been described with long-term therapy in patients with chronic congestive heart failure, in that higher ANP levels occur at lower atrial pressures and that ANP no longer decreases normally in response to acute decreases in atrial pressure (Rouleau et al., 1988). It is possible that captopril exerts certain beneficial effects by a relative increase of ANP, especially its reported natriuretic effect. However, further studies are required to substantiate this hypothesis.

Inhibition of local and systemic renin-angiotensin systems by captopril

On the basis of the foregoing data, it is clear that inhibition of angiotensin II synthesis, local and systemic, by angiotensin converting enzyme inhibitors is the major mechanism of action in the therapeutic effect of captopril. However, the relative importance may vary depending on the clinical or experimental situation. There is no doubt that inhibition of systemic angiotensin II is of crucial importance in the acute phase of congestive heart failure and hypertension, especially renovascular hypertension. There is also strong evidence that in chronic hypertension the local vascular renin-angiotensin system is the primary tar-

get for the therapeutic effect, with perhaps an important contributory role of the cardiac renin-angiotensin system in the prevention and regression of left ventricular hypertrophy (Dzau, 1988b). Inhibition of both local and systemic renin-angiotensin systems is also important in chronic heart failure, but this is less clearly defined.

An important contribution to this beneficial effect may be the inhibition of angiotensin II-mediated facilitation of noradrenaline release (Zimmerman, 1984; van Zwieten, 1986) , either locally in the heart or systemic. This will be discussed in more detail under paragraph 4 in the current chapter. Second, interference with the local vasculature may determine the different patterns of redistribution of regional blood flow, which may contribute to the symptomatic and functional improvement in patients with congestive heart failure (Leier, 1988). Third, the potential of captopril to attenuate progressive ventricular enlargement and hypertrophy following myocardial damage (Gavras, 1988b) may be partly due to inhibition of vascular angiotensin II. Finally, it has been shown that angiotensin converting enzyme inhibitors increase arterial caliber and distensibility, which may be important in promoting regression of cardiac hypertrophy, reducing hypertensive vascular injury and preventing tissue injury (Dzau, 1988c).

An important mechanism of action in the beneficial effects of captopril on ischemia-reperfusion induced myocardial infarction appears to be the inhibition of the generation of intracardiac angiotensin II. Direct evidence for cardiac angiotensin converting enzyme inhibition was provided by the demonstration that angiotensin converting enzyme inhibitor treatment attenuated local angiotensin I conversion and reduced angiotensin converting enzyme activity (Xiang et al., 1985). Furthermore, pretreatment with ramipril in vivo abolished angiotensin I-induced effects, but not those of angiotensin II (Linz et al., 1987). When added to the perfusate, angiotensin II enhanced postischemic reperfusion arrhythmias with reduction in cardiac and metabolic function. Other studies, carried out in rabbits, have demonstrated that large doses of exogenous angiotensin II can cause multifocal microscopic myocardial necrosis (Gavras et al., 1975). In vivo, inhibition of the synthesis of plasma angiotensin II will further contribute to these beneficial effects by reduction of afterload and myocardial oxygen demand. All these experiments suggest that both local and systemic generation of angiotensin II play an important role in the deleterious events which occur during ischemia and reperfusion, and consequently in the beneficial effects of captopril. However, inhibition of angiotensin II is not the only mechanism which appears to be involved in these effects. We could not detect any angiotensin II in the coronary effluent during ischemia and reperfusion in the untreated rat hearts (Appendix 3). Furthermore, the abolition of the effects when indomethacin was added to the coronary perfusate strongly suggests other, angiotensin-independent mechanisms (Appen-

dix 4; Li and Chen, 1987). This will be discussed more in detail under paragraph 2.

The same applies to the mechanisms of action which are involved in the effects of captopril on coronary blood flow. Angiotensin II has a well-documented potency as a coronary vasoconstrictor (Koch-Weser, 1984) and therefore interference with its generation by the vascular endothelium may influence coronary hemodynamic parameters. However, our experiments in the isolated rat heart strongly suggest that other, bradykinin-dependent and sulfhydryl-dependent mechanisms can also be of prime importance (van Gilst et al., 1987; van Gilst et al., 1988). This may change in vivo, where inhibition of cardiac and systemic angiotensin II may be the most important reason why captopril has a coronary vasodilatory effect, as has been described under conditions with activation of the renin-angiotensin system. A complex interplay between several mechanisms of action may explain why captopril and other angiotensin converting enzyme inhibitors can be beneficial in the situation of angina pectoris.

2. KININ-KALLIKREIN SYSTEM

As far back as 1968 it was suggested that the same enzyme which converts angiotensin I into angiotensin II also promotes breakdown of bradykinin (Bakhle, 1968). It was biochemically proven that this angiotensin converting enzyme is identical to kininase II, which catalyzes the hydrolytic removal of a carboxyl terminal depeptide from kinins leading to the inactivation of bradykinin (Yang et al., 1971). Consequently, inhibition of this enzyme by inhibitors such as captopril will lead to less degradation and thus to potentiation of its effect. The important question arises whether this interference with the kinin-kallikrein system plays a role in the mechanism of action of captopril. To address this question, it is first necessary to summarize the present knowledge of formation and breakdown of bradykinin (Regoli and Barabé, 1980; Carratero and Scicli, 1981; Douglas, 1987). This is shown in Figure 3.

Kinins are formed in biological fluids by enzymatic activation of naturally occurring peptides known as kininogens or kallikrein substrates which are present in plasma α2-globulin fraction. The greatest interest is directed towards the kallikreins, a group of enzymes of high substrate specificity which are present in plasma and in many other body fluids, cells and tissues (e.g. the kidney, various exocrine glands and their secretions, lymph, exudates, joint fluids). Most of the pre-kallikreins are present as an inactive form. Plasma kallikrein releases the nonapeptide bradykinin directly from a kininogen of high molecular weight (100,000). Glands and other tissue kallikrein release the decapeptide lys-bradykinin from kininogen of low (50,000) molecular weight. Bradykinin is quickly broken down by kininase II and by kininase I, the latter a slower-acting enzyme.

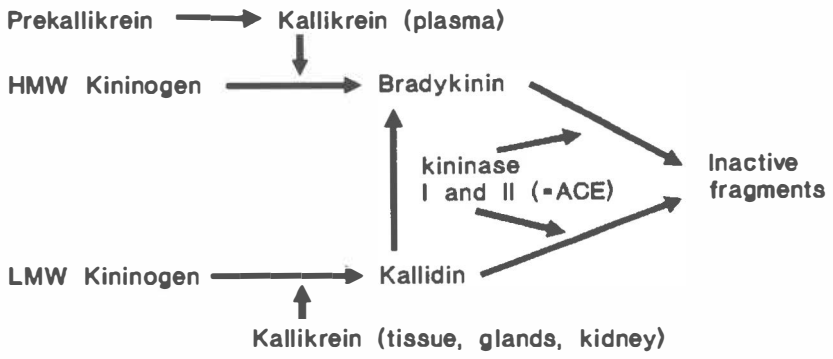

Figure 3. Components of the kinin-kallikrein system. Captopril inhibits kininase II, which is identical with the angiotensin converting enzyme.

Under normal conditions kinins are present in the circulation only in very low concentrations, their low levels being the result of the balance between simultaneous production and inactivation. Kinins have a half-life of only about 15 seconds. This has severely complicated studies on the role of bradykinin in normal and pathophysiological states. In addition, the measurement of blood levels is also limited by in vitro activation of kallikrein, leading to formation of bradykinin. Therefore, determination of plasma levels has been of little value in evaluating the role of bradykinin in cardiovascular disease.

Despite these facts, kallikrein and kinin have been associated from the moment of their discovery with the regulation of the cardiovascular system. Initially, kinins were associated with the development of pain following myocardial ischemia due to their nociceptive effect (Regoli and Barabé, 1980). More recently, focus has mainly been directed on the effect of bradykinin on vascular tone, especially of the coronary vasculature. On a molar basis, kinins are about ten times more potent than histamine in causing vasodilation. Two separate mechanisms of action appear to be involved.

The first mechanism relates to the release of prostaglandins, especially prostaglandin I2. Bradykinin is one of the first peptides known that have been shown to activate phospholipase A2, leading to production of arachidonic acid and subsequent synthesis of prostaglandins. In 1975 Needleman et al. showed that bradykinin decreases coronary vascular tone dose-dependently, accompanied by a dose-dependent release of prostaglandins (Needleman et al.,1975). Further evidence for a prostaglandin-mediated vasodilator effect of bradykinin was obtained when indomethacin prevented both coronary vasodilation and prstaglandin release (Moore et al., 1981; Swartz and Williams, 1982). Although these prostaglandins were originally thought to be PGE-like, further studies showed that the major prostaglandin-like substance released

from the heart after stimulation was prostaglandin I2 or prostacyclin (Needleman and Kaley, 1978). However, this inhibiting effect of indomethacin did not block the vasodilating effects of bradykinin and already in 1979 it was shown that an additional prostaglandin I2-independent mechanism had to be present (Schrör et al., 1979).

The second mechanism of action appeared to be the release of a so-called endothelium-derived relaxing factor (EDRF). EDRF was first described by Furchgott, who discovered that the vasodilating action of acetylcholine required the presence of endothelial cells through the release of a substance that caused relaxation of the vascular smooth muscle cell (Furchgott and Zawadski, 1980).

Subsequently it was discovered that several other peptides, among which bradykinin, were able to stimulate the release of EDRF (Cherry et al., 1983). EDRF, which is probably identical to nitric oxide (Palmer et al., 1987; Ignarro et al., 1987), appears to act on smooth muscle by stimulating guanylate cyclase and thereby the accumulation of cyclic GMP, the ultimate mediator of vasodilation (Ignarro et al., 1987). Recently, it was shown in the isolated guinea pig heart that the endogenous formation of nitric oxide in the heart is quantitatively sufficient to influence the coronary vasculature (Kelm and Schrader., 1988). Thus, EDRF may play a significant role in the regulation of coronary vascular resistance.

Release of EDRF and prostaglandin I2 by the endothelial cell appears to express two separate mechanisms of action by bradykinin, possibly mediated by activation of two subtypes of B1 and B2 receptors (Toda et al., 1987). B1 receptor subtypes can not only be found on the endothelial cell but also on the smooth muscle cells of the vasculature, which also play a role in the release of prostaglandin I2. Furthermore, a direct effect via these receptors on smooth muscle cells has been described (Beny et al., 1987). It is evident that there is a heterogeneity in the mechanisms of action of bradykinin in blood vessels from the same species (coronary vs. renal and mesenteric) and in the same vessel from different species (Toda et al., 1987). To complicate things further it has been shown that kinin can stimulate renin secretion from the kidney and vasopressin from the central nervous system, as well as induce catecholamine release from the adrenal medulla and sympathetic ganglia (Benetos et al., 1986). These different mechanisms and interactions with other regulatory systems, which depend on species, type of vessel and concentration, are responsible for the fact that the vascular effects of the kinin-kallikrein system are variable and sometimes contradictory, producing contraction, dilation or biphasic responses (Benetos et al., 1986).

From the very beginning, kallikrein and kinin have been associated with the regulation of the cardiovascular system. Little is still known about its exact role in cardiovascular disease, especially hypertension and ischemic heart dis-

ease (Carratero and Scicli, 1981). Although many authors have attributed a role for bradykinin in blood pressure regulation under various conditions, this has been very difficult to prove, mainly due to the above-mentioned problems of accurately estimating the plasma and tissue levels. Only recently, with the help of selective bradykinin antagonists (Vavrek and Stewart, 1985), it has been demonstrated in hypertensive rats that bradykinin is involved in the maintenance of normal blood pressure and could be a major counteractive mechanism to other vasoconstrictor systems (Gavras et al., 1987).

On the basis of the hypotensive effects of bradykinin, it was initially assumed that the kallikrein-kinin system was involved in the hypotensive response of angiotensin converting enzyme inhibitors. This involvement turned out to be more difficult to prove than originally thought, although it was demonstrated that captopril could potentiate exogenous bradykinin (Mullane et al, 1980). Circulating bradykinin levels in both animals and humans were reported to increase only transiently, to remain the same or even to reduce, after captopril was given (Johnston et al., 1982). Studies with concomitant administration of indomethacin, aimed at blocking the bradykinin mediated hypotensive effect of prostaglandins, showed conflicting results (VanDongen et al., 1982; Moore et al., 1981; Quilley et al., 1987).

The question was raised whether or not the circulatory bradykinin is of any importance in the regulation of cardiovascular system. Subsequently, it was found that captopril increased urinary kinin concentration without a change in circulatory bradykinin levels, suggesting an increase in local tissue concentration of kinin, especially in the kidney (Zusman, 1987). This theory of interference with the tissue renin-kallikrein system was strengthened by the presence of kinin forming enzymes in the vascular wall (Regoli and Barabé, 1980). Furthermore, it was demonstrated that angiotensin converting enzyme inhibitors induced long-lasting inhibition of kininase activity in the arterial wall (Lindsey et al., 1987). Initial studies with aprotine and bradykinin antibodies showed that a significant part of the antihypertensive effect of captopril seemed to be mediated by kinins (Mimram et al., 1980; Carratero et al., 1981). Further studies with a bradykinin antagonist demonstrated that the blood pressure lowering effect of angiotensin- converting enzyme inhibitors can be partly antagonised by bradykinin antagonists, but not completely (Carbonell et al., 1988; Benetos et al., 1986). Therefore, angiotensin converting enzyme inhibitors appear to exert their antihypertensive effect in part through potentiation of bradykinin. The relative importance of this effect may vary, depending among others on the presence or absence of an activated renin-angiotensin system.

What about the role of bradykinin in the heart and especially in ischemic heart disease? Interestingly, in 1973 it was shown that after experimental ligation of the left anterior descending coronary artery, a marked elevation of the bradykinin level, a slight decrease of the bradykininogen level and a mild in-

crease of bradykinininase occurred in the coronary sinus blood (Kimura et al., 1973). These results suggested that the production of bradykinin was activated in ischemic myocardial tissues. This was in accordance with earlier experiments, which indicated activation of plasma kinins following myocardial infarction and angina pectoris. It was suggested that this increase in bradykinin was responsible for chest pain due to myocardial ischemia, since kinins stimulate pain receptors. The same authors reported a study of 23 patients with acute myocardial infarction of whom 7 died (Kimura et al., 1975). They noted a decrease in kininogen with a simultaneous increase in bradykinin. These changes correlated significantly with the decrease in blood pressure and total peripheral resistance. Moreover, bradykinin levels were significantly lower in the lethal cases than in the surviving ones. They concluded that the release of kinin at the local myocardial site may perhaps be a beneficial defensive mechanism contributing to survival (Torstila, 1978). The above-mentioned demonstration of Needleman et al. that bradykinin is a potent stimulator of prostaglandin synthesis in the heart (Needleman et al., 1975) corroborates this. Reflex bradycardia will decrease cardiac oxygen demand and coronary dilation will increase oxygen supply.

These promising data were not followed by further elucidation of the role of the kinin-kallikrein system in heart disease, despite the fact that stimulation of prostaglandin synthesis was considered to have a beneficial role both in patients with angina pectoris and severe heart failure, which could be blocked by indomethacin (Friedman et al., 1981; Dzau et al., 1984). Again, as with hypertension, this was mainly due to problems with the bradykinin assay. In one of the first studies in patients with severe congestive heart failure, no change in plasma kinin could be detected even after administration of captopril (Dzau et al., 1979). Already then it was postulated that it is not the circulating kallikrein system which is of crucial importance, but the local, intracardiac formation of bradykinin.

The importance of this local cardiac generation of bradykinin is further supported by our experiments and those of Schölkens and co-workers in isolated rat hearts. Indomethacin completely abolished the beneficial effects of captopril on reperfusion arrhythmias and myocardial damage after reversible ligation of the left coronary artery (Appendix 4), indicating an important role for prostaglandins as possible mediators of the action of angiotensin converting enzyme inhibitors. When bradykinin was added to the perfusate fluid during reperfusion, incidence and duration of the reperfusion arrhythmias were markedly reduced and this was further improved by ramipril pretreatment (Linz et al., 1987). Furthermore, bradykinin perfusion restored the respective tissue levels of glycogen, lactate, ATP and creatine phosphate in both control and ramipril pretreated ischemic hearts to values comparable with freshly prepared hearts before perfusion, indicating a beneficial effect on ischemia-reperfusion

injury (Linz et al., 1987). Experiments with ramipril on the coronary flow showed an increase in coronary blood flow, correlating with an increase of 6-keto-PGF1α, recovered from the isolated rat heart (van Gilst et al., 1987).

Although these experiments give no definite proof and are partly based on on the assumption that the effect on prostaglandin I2 is bradykinin-mediated, they strongly suggest that potentiation of intracardiac bradykinin can play an important role in the mechanism of action of converting enzyme inhibitors. This is further corroborated by ongoing experiments with bradykinin antagonists. The antagonist abolished the cardioprotective effects of ramipril in the isolated rat heart during ischemia and reperfusion (Schölkens et al, 1988b) and antagonized the flow increase of enalaprilat (Tio et al., 1987). Furthermore, in Braun-Norway rats without high molecular weight kininogen, no beneficial effects of ramipril were detected on reperfusion-induced arrhythmias or on myocardial damage (Schölkens et al., 1988b).

All these results strongly suggest that locally generated bradykinin can play an important role in ischemic heart disease. Although only the angiotensin converting enzyme, identical to kininase II, has been demonstrated in both the endothelial cell of the coronary vasculature and the myocardium, one may speculate whether an entire cardiac bradykinin-kallikrein system is present in the heart. A strong parallel to the local renin-angiotensin system can be drawn. A circulating precursor peptide (kininogen vs. angiotensinogen), which is activated intracellularly by an enzyme (kallikrein vs. renin), is present in large concentrations in the serum in an inactive form (pre-kallikrein vs. pro-renin).

Interestingly, it has been demonstrated that both plasmin, which is involved in the conversion of pre-kallikrein to kallikrein, and kallikrein itself can activate pro-renin to renin (Derckx, 1987). Activation of plasmin by thrombolysis with streptokinase will therefore not only stimulate the kinin-kallikrein system but also the renin-angiotensin system. Concomitant administration of captopril will further enhance the (vasodilating) effects of bradykinin while it will inhibit the (vasoconstrictive) effects of angiotensin II. This may explain why severe hypotension occurred when streptokinase was given at the same time as captopril (Appendix 7). Clearly, more than one link exists between the kinin-kallikrein system and the renin-angiotensin system.

Further studies will have to demonstrate tissue kallikrein and kininogen messenger RNA, i.e. gene expression, in the heart in order to prove the presence of a local kinin-kallikrein system. If this system is really active in the heart, it might be one of the systems designated to counteract the effects of the local renin-angiotensin system. Changes of the balance between these two systems may play a crucial role in the development of heart disease. The beneficial role of captopril, as described in this thesis, may be largely due to a restoration of this balance.

3. PROSTAGLANDIN SYSTEM

Although it has long been known that the heart can produce prostanoids (Needleman et al., 1975), until recently the coronary vasculature was considered the only important local site of prostanoid biosynthesis (Kraemer et al., 1976; Needleman and Kaley, 1978)). However, recent evidence has demonstrated the synthesis of prostaglandins E2, F2α, I2 and thromboxane A2 (Escobar et al.,1983; Mehta and Mehta, 1985) in myocardial tissue. These may play an important role in the control of the circulation. However, the exact contribution of cardiac prostanoids to the function of the normal and ischemic heart has not been clearly resolved.

The main attention has been focused on the effect of prostaglandins on the coronary vasculature in ischemic heart disease. Several authors have suggested that prostaglandin and thromboxane synthesis may counteract each other in the normal regulation of coronary blood flow (Needleman and Kaley, 1978). In other words, prostaglandin I2 synthesized in coronary vascular tissue serves as a physiological antagonist of thromboxane A2 which is synthesized by the platelets. In patients with ischemic heart disease, an increase in thromboxane A2 may predispose to acute events like myocardial infarction, whereas increase of prostaglandin I2 counteracts this effect by its vasodilating properties. When indomethacin was given to patients with coronary artery disease, a significant reduction of coronary sinus blood flow was seen, indicating a vasodilating effect of prostaglandin I2 (Friedman et al., 1981). On the other hand, pharmacological intervention with aspirin, aimed at selective inhibition of thromboxane A2, appears to beneficially influence the development of ischemic heart disease (Physicians Health Study Research group, 1988).

Little is known about the role of prostaglandins in ischemic injury and subsequent reperfusion. Animal experiments have demonstrated that exogenous prostaglandin I2 favorably modifies cellular injury evoked by regional or global reduction of blood flow, with reduction of the incidence of severe arrhythmias and total purine overflow upon reperfusion (van Gilst, 1986). The precise mechanism remains unknown, but inhibition of catecholamine release appears to play an important role. Finally, vasodilatory prostaglandins, such as prostaglandins I2 and E2, play a critical part in preserving circulatory function in vasoconstrictive states, especially in severe congestive heart failure. This is demonstrated by the hemodynamic deterioration which occurs when indomethacin is given to these patients (Dzau et al., 1984).

Angiotensin converting enzyme inhibitors can influence cardiovascular prostanoids in several ways, both by direct and indirect mechanisms. In vitro, captopril stimulated prostaglandin E2 synthesis by renomedullary interstitial cells, which was not seen after enalaprilat (Zusman, 1987). It was suggested

that this was due to a direct, sulfhydryl-related effect, possibly also relevant in vivo. This awaits further confirmation.

Complex interrelations exist between the renin-angiotensin system, the sympathetic nervous system, the kallikrein-kinin system and prostaglandin metabolism (Needleman et al., 1975; Nasjletti and Malik, 1979; Zusman, 1987). This is shown in Figure 4. Since captopril and other angiotensin converting enzyme inhibitors interfere with levels of both angiotensin II and bradykinin and attenuate sympathetic nerve activity, indirect changes in prostaglandin metabolism may also occur which explain some of the clinical effects of these drugs (Swartz et al., 1980; Swartz and Williams, 1982). However, these changes are not consistent and may vary depending on the activation of these systems and their contribution to the (patho)physiological state. For instance, the inhibitory effect of indomethacin on the antihypertensive effect of captopril appears to depend on the renin status and sodium balance (Moore et al., 1981; Zusman, 1984). The effect of captopril on ischemia-reperfusion injury, when prostaglandin metabolism is increased, could be abolished by indomethacin in the isolated rat heart (Appendix 4). In contrast, in the nonischemic situation no inhibitory effect was seen on the captopril mediated increase in coronary blood flow (van Gilst et al., 1987). Finally, observations are further obscured by the fact that both angiotensin converting enzyme inhibitors and regulatory systems interfere with non-cardiovascular prostanoids, especially in the kidney.

The contribution of prostaglandins to the beneficial effects of captopril and other angiotensin converting enzyme inhibitors in heart disease remains to be established. However, the experiments with indomethacin in the isolated rat heart strongly suggest that facilitation of myocardial prostaglandin I2 synthesis

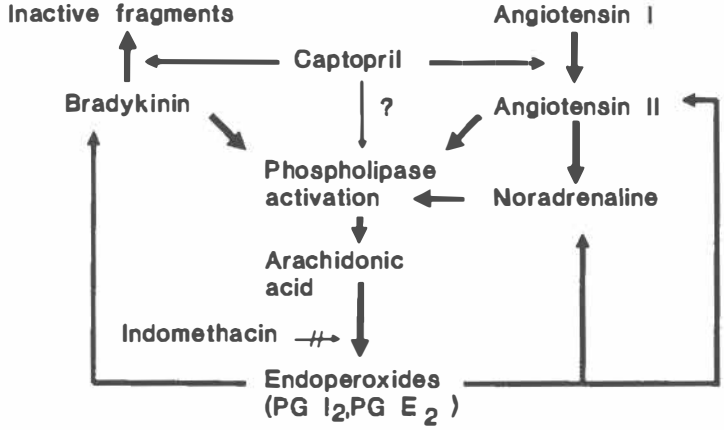

Figure 4. Effects of captopril on prostaglandin metabolism.

plays an essential part in the protective effects of these drugs during ischemia and reperfusion (Appendix 4). This is also indicated by other investigators in similar experiments, where the reduction of CPK levels after coronary occlusion by captopril was abolished by pretreatment with indomethacin and could be mimicked by prostaglandin I2 infusion in untreated rat hearts (Li and Chen, 1987). The facilitation of prostaglandin I2 synthesis is also shown by two studies of the group of Düsing, which demonstrate a direct stimulatory effect of captopril and ramiprilat on prostaglandin I2 synthesis in vascular tissue (Düsing et al., 1983; Scherf et al., 1986). Interestingly, the effect by ramipril could be attenuated by aprotinin, a potent kallikrein inhibitor, suggesting that the stimulatory effect appears to be dependendent on an intact kinin system (Scherf et al., 1986).

These results demonstrate that angiotensin converting enzyme inhibitors can interfere with arachidonic acid metabolism. Eventually this may result in beneficial effects in conditions where arachidonic acid metabolism is increased, such as hypoxic conditions. However, captopril can have more than one effect on arachidonic acid metabolism and interferes not only with the cyclo-oxygenase pathway, but also with the lipo-oxygenase pathway. This was shown in the nonischemic situation where FPL 55712, a leukotriene (LTC4 and D4) antagonist, potentiated the coronary dilatory effect of captopril (van Gilst et al., 1987). It was speculated that the sulfhydryl-moiety of captopril was responsible for this effect, but this needs further confirmation.

One must be careful to extrapolate the promising in vitro results to the clinical situation. Other regulatory systems are active in vivo, especially thromboxane A2, which will counteract the effects of captopril on PGE2 and prostaglandin I2. This is supported by a study with captopril in patients with essential hypertension which indicated that thromboxane A2 counteracted the hypotensive effect of captopril (Kudo et al., 1988). The same applies to other counterregulatory substances such as angiotensin II, noradrenaline and vasopressin. However, a dissociation may occur, leading to a sustained increase of especially prostaglandin I2 and E2. This was recently shown in patients with congestive heart failure secondary to chronic artery disease, who demonstrated a dissociation between the renin-angiotensin system and prostaglandin E2 during captopril therapy (Dzau and Swartz, 1987c). Therefore, captopril can reinforce the effects of certain prostaglandins, which may favorably influence the clinical outcome in certain disease states.

4. SYMPATHETIC NERVOUS SYSTEM

Cardiovascular hemostasis is under the regulatory control of the autonomic nervous system, which closely collaborates with the renin- angiotensin system in the maintenance of myocardial function. Although an inhibitory effect of

angiotensin II on vagal transmission has been described, main attention has been focused on the interaction between the renin-angiotensin system and the sympathetic nervous system. These systems have an active and supportive relationship. On the one hand angiotensin II facilitates noradrenaline release through presynaptic receptor mechanisms, promotes vasoconstriction directly through a postsynaptic receptor, blocks neuronal uptake of noradrenaline, enhances noradrenaline biosynthesis in the adrenergic nerve terminal, releases adrenaline from the adrenal medulla, acts on the adrenal cortex to release aldosterone, augments the vascular smooth muscle response to various agonists and probably stimulates central noradrenaline activity as well (Zimmerman 1981; Zimmerman 1984; van Zwieten 1986). These pre- and postsynaptic facilitory effects of angiotensin II and inhibition by captopril are depicted in figure 5. On the other hand, the release of renin in the kidney, and possibly in other organs, is under direct and indirect control of the sympathetic nervous system. Although closely related, the interaction between the two systems is a substitutive one in the sense that one system backs up the other rather than modulating it (Zimmerman, 1984; van Zwieten, 1986). However, it is clear that by interfering with the renin-angiotensin system system captopril and other angiotensin converting inhibitors will influence the function of the autonomic ner-

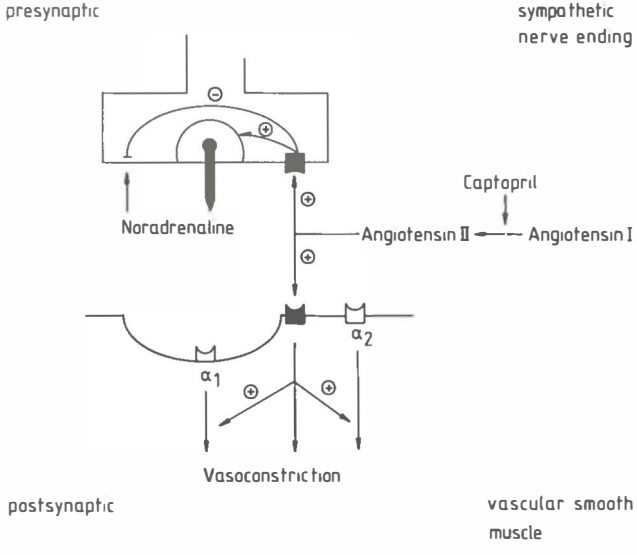

Figure 5. Pre- and post-synaptic facilitory actions of angiotensin II on neurogenic vasoconstriction. + = potentiating effect; − = inhibitory effect. Captopril inhibits both pre- and postsynaptic effects.

vous system. These changes may explain some of the pharmacological properties of these drugs, which have important implications.

Increased parasympathetic activity, in combination with failure of noradrenaline to rise despite marked hypotension, may explain why no reflex tachycardia occurs and heart rate even decreases following converting enzyme inhibition (Osterziel et al., 1988, Appendix 1). Furthermore, this reduced sympathetic activity may be one of the reasons why during chronic therapy in patients with congestive heart failure the therapeutic response persists, in contrast to some other vasodilating agents (Kluger et al., 1982; Daly et al., 1986). One may also speculate whether the improved survival, which has been reported after treatment with angiotensin-converting enzyme inhibitors, is in some way related to this inhibition since noradrenaline itself is associated with a poor prognosis in these patients (Cohn et al., 1984). Moreover, the reported arrhythmogenic effects of angiotensin II, which are associated with the poor prognosis of these patients, can be mediated by noradrenaline since angiotensin II causes a facilitation of noradrenergic neurotransmission in the heart (Dzau, 1988a). This may explain the reduced inducibility of sustained ventricular tachycardia at programmed electrical stimulation during the chronic phase of acute myocardial infarction (de Langen et al, 1989).

Reduction of noradrenaline also appears to play an important role in the beneficial effect of captopril on ischemia-induced reperfusion arrhythmias. Both in the isolated rat heart, in the closed-chest pig model, and in patients undergoing thrombolysis, administration of captopril resulted in a significant reduction of noradrenaline levels (Appendices 3, 5, and 7). In the isolated rat heart the question remains whether the reduction in noradrenaline overflow is dependent on the local inhibition of angiotensin II or whether other mechanisms are involved (Appendix 3). It has been described that the overflow of noradrenaline from isolated perfused rabbit hearts during stimulation of the sympathetic nerves was enhanced by low concentrations of angiotensin II (Starke et al., 1971). Pretreatment with two other angiotensin converting enzyme inhibitors, enalapril and ramipril, markedly mitigated the hemodynamic response following electrical sympathetic stimulation of the cardiac nerves in vitro, again suggesting a modulation of noradrenergic transmission by inhibition of local angiotensin II (Xiang et al., 1985). These findings are corroborated by the observation that ramipril inhibits noradrenaline release in rat hearts with intact sympathetic innervation perfused retrogradely in situ (Mayer et al., 1986).

However, angiotensin II was below the detection limit (2 pg/ml) in the coronary effluent in our experiments in the isolated rat heart, both in normoxic conditions and during ischemia and reperfusion (Appendix 3). Also, one must keep in mind that most experiments on the interference of captopril with angiotensin II-mediated noradrenergic transmission have been performed in pi-

thed rats or isolated vascular tissue subjected to noradrenaline. Therefore, other mechanisms may contribute to the beneficial effects we observed in the isolated rat heart on ischemia-induced reperfusion injury. At higher doses an angiotensin-independent action of captopril has been reported leading to an attenuating effect on the vascular reponse of noradrenaline (Collis and Keddie, 1981), but this does not explain the reduction of overflow. Our experiments strongly suggest that the reduction of catecholamine overflow is at least partly mediated via a stimulation of prostaglandin I2 synthesis, which can be induced by captopril either directly or mediated by bradykinin (Appendix 4). The marked reduction by captopril of the overflow of noradrenaline appears to be related to a complex interaction with the local renin-angiotensin system, the kallikrein-kinin system and prostaglandins.

Another question is the relevance of this attenuating effect by captopril on sympathetic nervous system activity. Our experiments in vitro strongly suggest that this plays a central role in the protective effects on reperfusion arrhythmias, although we did find a discrepancy between the dose-dependency of the effects on arrhythmias and cellular damage and the complete reduction of noradrenaline overflow (Appendix 3). Further support for this role is the concomitant abolishment of both phenomena when indomethacin was added (Appendix 4). However, an epiphenomenon cannot be ruled out. Also speculative is the clinical importance of the reduction of noradrenaline efflux upon reperfusion in the in vivo studies, both in the closed-chest pig model and in patients undergoing thrombolysis (Appendices 5 and 7). Numerous reports have demonstrated stimulation of the sympathetic nervous sytem after myocardial infarction (McDonald et al., 1969; Videbaek et al., 1972; Karlsberg et al., 1981; McAlpine et al., 1988). A consistent finding in the literature has been the association of very high catecholamine levels with the presence of left ventricular failure, shock or ventricular tachycardia and fibrillation (McAlpine et al., 1988). This stimulation of catecholamines increases myocardial oxygen consumption by positive inotropy and chronotropy, reduces collateral flow by coronary vasoconstriction, and augments afterload by peripheral vasoconstriction. All these effects will tend to increase infarct size and the likelihood of ventricular fibrillation. This may explain why early treatment with a beta-blocker can be beneficial in this situation (Yusuf et al., 1988). During subsequent chronic treatment, the attenuating effect of captopril on catecholamine levels may lead to a reduction of ventricular arrhythmias. This may be one of the reasons why regression of left ventricular hypertrophy and attenuation of ventricular enlargement due to hypertension can occur in the late convalescent phase of myocardial infarction (Cobbe, 1987). If the beneficial effects of captopril on myocardial damage following ischemia and reperfusion are confirmed by further large-scale studies, this will substantiate the importance of the interaction between captopril and the sympathetic nervous system.

Finally, inhibition of the sympathetic baroreceptor reflex may also play a role in the antianginal effect which has been found with captopril. Angiotensin converting enzyme inhibitors have been shown to decrease hypoxia-mediated noradrenaline release (Xiang et al., 1985), which contributes to the reduction of oxygen demand. This makes captopril a potential anti-anginal agent in patients with concomitant high noradrenaline levels, such as patients with angina pectoris in combination with congestive heart failure, despite the fact that systemic hypotension can occur. This will be discussed further in chapter IV.

5. SULFHYDRYL GROUP

The active site of the angiotensin converting enzyme contains zinc, which plays an active role in the catalytic process and has a dominating influence on the binding affinity of angiotensin- converting enzyme inhibitors. Therefore, during the development of oral angiotensin converting enzyme inhibitors main attention has been concentrated on strengthening this interaction with the zinc atom (Ondetti et al., 1977). For this reason captopril contains a sulfhydryl group which dramatically increases its binding potency with the angiotensin converting enzyme. However from the beginning, this sulfhydryl group was associated with certain side-effects, such as rash, taste disturbance, neutropenia and immune complex glomerulopathy. These adverse reactions formed a distinctive pattern, which was also seen with other sulfhydryl-containing compounds, such as penicillamine (Jaffe, 1986). Although later studies demonstrated that in lower dosages and with more careful patient selection these side effects were markedly reduced (Gavras,1988a), the sulfhydryl group was put in an unfavorable light. Subsequently, in newer developed angiotensin converting enzyme inhibitors the sulfhydryl group was substituted for other binding sites. At the lower doses now recommended, only taste disturbance and skin rash are convincingly more frequent in patients treated with captopril when compared to enalapril.

Due to this negative role of the sulfhydryl-group, only few studies have addressed the question whether this thiol moiety may also exert beneficial effects. Because of the "penicillamine-like" side effects, rheumatologists have studied the drug for its potential usefulness in a limited number of patients with rheumatoid arthritis. Not only did a favourable response occur (Martin et al., 1984), but also evidence was obtained that it was indeed the sulfhydryl-group in captopril that was responsible for its anti-rheumatoid activity (Jaffe, 1984). However, these studies await further corroboration and captopril has as yet no established place in the treatment of rheumatoid diseases.

On a theoretical basis one can postulate that the sulfhydryl-group can be responsible for at least three special cardiovascular actions, namely binding of free radicals, potentiation of nitrates and a coronary vasodilating effect.

A. The binding of free radicals.

During recent years much attention has been focused on the role of oxygen-derived free radicals in the tissue damage following ischemia and reperfusion. Increasing evidence suggests that the generation of free radicals occurring within the first few minutes of reperfusion may play an important role in reperfusion induced depression of contractility and reperfusion arrhythmias (van Gilst, 1986; Braunwald and Kloner, 1986; Becker and Ambrosio 1987; Southorn, 1988). These activated oxygen species, including superoxide anion, hydrogen peroxide and hydroxyl radicals, are formed as intermediates during the univalent reduction of oxygen to water, in, under normal conditions, small quantities. However, during ischemia biochemical changes occur, which are thought to be the basis for a burst of production of free radicals on reintroduction of molecular oxygen at reperfusion.

Based on this theory of free radical induction of ischemia-reperfusion injury, several studies in animal experiments have proposed that limitation of free radical damage can beneficially influence the detrimental effects on the heart (van Gilst, 1986). Cumulative data from experimental preparations of regional and global myocardial ischemia and reperfusion have substantiated that free radical scavengers, such as the enzymes superoxide dysmutase and catalase, which degrade superoxide anion and hydrogen peroxide, have a beneficial effect on myocardial cell damage (Maza and Frishman, 1987). This was also shown for allopurinol, an inhibitor of xanthine oxidase which is responsible for the formation of free radicals (Southorn, 1988). Due to the presence of a sulfhydryl group captopril can also scavenge free radicals in a comparable manner to sulfhydryl-containing compounds. This was shown in a recent study in three different in-vitro systems (Westlin and Mullane, 1988).

The question remains how important this free radical scavenging is in the beneficial effects of captopril on myocardial injury. It may explain why part of the beneficial effects also occur when captopril was given just before reperfusion (Appendix 6). Improvement of regional myocardial function was found when the inactive stereoisomer SQ 14.534 was given before reperfusion, whereas enalaprilat, given from the start of the experiment, did not show any effects (Westlin and Mullane, 1988). In contrast, reduction of ventricular fibrillation only occurred following administration of enalaprilat. In the isolated rat heart, ramiprilat, which does not contain a sulfhydryl group, exerts similar beneficial effects to captopril, even when given just before reperfusion (Appendix 4; Linz et al., 1987). This effect was shown to be dependent on the angiotensin converting enzyme. In contrast, enalaprilat only reduced purine overflow but did not influence reperfusion arrhythmias and hemodynamics (Appendix 4).

The exact role of free radical scavenging in the beneficial effects of captopril on reperfusion injury remains to be established. If captopril could be used as a free radical scavenger, this may have important clinical implications not only in the setting of acute myocardial infarction, but also in other clinical situations in which generation of free radicals plays an important role, such as circulatory shock.

B. Potentiation of nitrates.

During the past century nitrates have become the most widely used agents in angina pectoris and congestive heart failure. The main action appears to be a consequence of their vasodilating effects on the systemic circulation, predominantly the capacitance veins. Recent studies have demonstrated that these vasodilator effects are mediated via activation of soluble guanylate cyclase, a process which probably involves a number of sulfhydryl-dependent steps, including denitration, formation of S-nitrosothiols as active intermediates, and possibly stabilisation of the activated guanylate cyclase (Needleman et al., 1973; Ignarro et al., 1981). Thus, nitrate-induced vasodilator responses may be modified by changes in sulfhydryl-availability. A deficiency of reduced sulfhydryl-groups during continued administration of nitrates may explain why their vascular effect are rapidly attenuated during sustained therapy, a phenomenon which is known as tolerance (Cowan, 1986). Further evidence for this hypothesis can be derived from studies with N-acetylcysteine, a sulfhydryl containing compound, which reverses the loss of vascular responsiveness (Torresi et al., 1985; Packer et al., 1987c).

On the basis of these findings, the theory can be put forward that captopril can potentiate the hemodynamic effects of nitrates due to the presence of the sulfhydrylgroup. This is shown in Figure 6. Our experiments in the isolated rat heart support this hypothesis (Appendix 8). Further clinical evidence can be derived from our study in patients with angina pectoris (Appendix 9), although mechanisms other than the potentiation of the nitrate therapy may contribute to the beneficial effects of captopril on exercise tolerance. If a beneficial interaction between captopril and nitrates is confirmed, it may have important clinical consequences since patients with ischemic heart disease often have decreased myocardial function, necessitating the use of an angiotensin converting enzyme inhibitor.

C. Coronary dilation.

As described above, sulfhydryl groups play an important role in the vascular smooth muscle relaxant response to nitrogen-oxide containing vasodilator drugs. This discovery, which was already described in 1973 (Needleman et al., 1973), has recently come into a new perspective when it was discovered that

the above-mentioned endothelium-derived relaxing factor is identical to nitric oxide (Ignarro et al., 1987; Palmer et al., 1987; Radomski et al., 1987). This could mean that the sulfhydryl-containing compounds not only potentiate the effects of exogenous nitrate-containing compounds, but also exert vasodilatory effects on their own through a potentiation of this endogenous vasodilating agent. In the case of captopril this effect may be further enhanced by its potentiation of bradykinin, which also stimulates the formation of EDRF (Cherry et al., 1982). This is shown in Figure 6.

This theory of a sulfhydryl mediated vasodilatory effect is supported by our experiments in the isolated rat heart on coronary blood flow (van Gilst et al., 1987; van Gilst et al., 1988). First, captopril and another sulfhydryl-containing angiotensin-converting enzyme inhibitor, zofenopril, induced a faster and much more potent coronary vasodilating effect than ramiprilat (Appendix 8). Second, three other sulfhydryl-containing compounds without angiotensin converting enzyme inhibiting properties, namely the inactive (R-S) isomer of captopril, glutathione and cysteine, all showed similar effects to captopril. Third, the administration of a bradykinin antagonist completely antagonized the vasodilating effects of enalaprilat, but only partly or not at all those of the sulfhydryl-containing angiotensin converting enzyme inhibitors (Tio et al., 1987). Therefore, captopril appears to exert vasodilatory actions not only by converting enzyme inhibition but also, and even much stronger, by the pre-

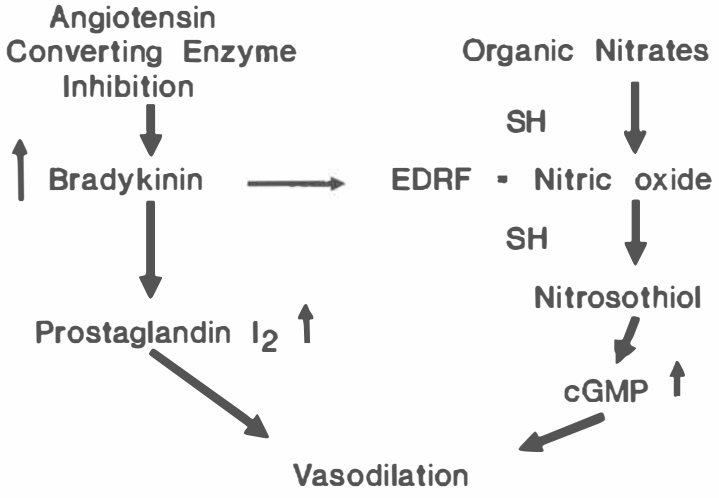

Figure 6. Relationship between angiotensin converting enzyme inhibition and the presence of a sulfhydryl (SH) group in the vasodilatory effects of captopril and its potentiation of organic nitrates. EDRF = endothelium-derived relaxing factor.

sence of its sulfhydrylgroup. Interestingly, this bradykinin potentiating activity was also demonstrated in vivo in the rat for the captopril disulphide dimer, which has no CE-inhibiting activity (Drummer and Kourtis, 1988). This may imply that the importance of the sulfhydrylgroup is not restricted to the in vitro situation, but may bear clinical relevance.

CHAPTER III

EFFECTS OF CAPTOPRIL IN EXPERIMENTAL MODELS

1. CONGESTIVE HEART FAILURE

Although it has been known for more than 40 years that stimulation of the renin-angiotensin system may occur in patients with congestive heart failure (Merill et al., 1946), the exact role of this system remains controversial. Levels of plasma renin and aldosterone in patients with cardiac failure have been reported as variously high, normal or even low (Dzau et al., 1981). Experiments in conscious animals with cardiac failure demonstrated that the renin-angiotensin system is activated during the acute phase after a reduction of cardiac output, which is essential for the maintenance of systemic arterial pressure (Davis, 1962; Watkins et al., 1976; Morris et al., 1976; Freeman et al., 1979). This leads to retention of sodium and water and extracellular volume expansion occurs if compensation is adequate. Eventually arterial and atrial pressure return to normal and plasma renin activity and plasma aldosterone fall to normal levels. Consequently, inhibition of the renin-angiotensin system with selective inhibitors (saralasin, teprotide, captopril) demonstrated that the blood pressure lowering effect declines with time (Watkins et al., 1976; Freeman et al., 1979). If compensatory mechanisms are inadequate and/or diuretic treatment is given, the renin-angiotensin system remains activated and a vicious circle ensues.

This persistent activation of the renin-angiotensin system, together with other neurohumoral systems, especially the sympathetic nervous system and vasopressin, will eventually be detrimental for the heart: peripheral resistance increases, leading to a reduction of cardiac output, retention of sodium and water, and subsequently to an increased left ventricular filling pressure pressure. The consequences are clinical signs and symptoms of congestive heart failure. Pharmacological blockade of the renin-angiotensin system may therefore reduce systemic vascular resistance and left ventricular filling pressure and increase cardiac output, which results in clinical improvement. This has been demonstrated in several animal models with teprotide, captopril and enalapril (Freeman et al., 1979; Shionoiri et al., 1987). Furthermore, it has been shown during atrial pacing that early treatment with captopril in anesthetized dogs effectively diminishes the deterioration of cardiac function during the development of congestive heart failure (Rieger et al., 1984).

It should be emphasized that the above-mentioned experiments have all

concentrated on the plasma renin-angiotensin system. As has been mentioned in the previous chapter, besides the systemic renin-angiotensin system a local renin-angiotensin system also exists in the blood vessels and the heart. This may play an important role in the pathogenesis of heart failure and in the effects of captopril and other angiotensin converting enzyme inhibitors. Based on the hypothesis that treatment with angiotensin converting enzyme inhibitors can beneficially influence the chain of events which leads to the development of congestive heart failure (Packer 1988), a number of important animal experiments have been carried out. These have been directed to two major causes of congestive heart failure, namely hypertension and ischemic heart disease.

In hypertension the heart adapts to the abnormal, sustained hemodynamic burden by an increase in mass. Initially, this will maintain systemic perfusion but, with prolongation of the pressure overload and progression of cardiac hypertrophy, ultimately left ventricular performance deteriorates. It has been shown in systemic hypertensive rats that treatment with captopril can produce a marked regression of cardiac hypertrophy (Sen et al., 1980; Pfeffer JM et al., 1982). Furthermore, in experiments in rats with left ventricular hypertrophy due to experimental aortic stenosis, angiotensin converting enzyme inhibitors reduced the development of left ventricular hypertrophy when given at an early stage and caused significant regression once left ventricular hypertrophy had developed (Sen et al., 1980; Linz et al., 1988). This prevention and regression of left ventricular hypertrophy can be associated with restoration of contractile reserve and coronary flow and with improvement of cardiac performance (Fouad et al., 1986). This may retard and possibly even prevent the development of congestive heart failure.

Myocyte hypertrophy also occurs following acute myocardial infarction in order to compensate for the loss of muscle mass and function (Anversa et al., 1985). However, if the loss of myocardium is greater than 20%, compensatory mechanisms will fail with increase of both systolic and diastolic wall stress, leading to ventricular dysfunction and enlargement (Pfeffer MA et al., 1979). This dilatation of the left ventricle, also called remodeling, is a continuous process starting within 24 hours after infarction with further progression during the chronic phase after scar tissue formation is complete. It has been demonstrated that the ultimate prognosis is related to the severity of the cardiac dysfunction (Bigger, 1986). Therefore, this progressive ventricular enlargement in combination with ventricular hypertrophy may be of major importance with regard to cardiovascular morbidity and mortality during the subacute and chronic phase of acute myocardial infarction. Reduction in workload and subsequent wall stress may reverse this process, leading to reduction of ventricular enlargement and consequently improvement of myocardial function and survival.

This was shown for the first time by two studies which demonstrated that treatment with captopril early after myocardial infarction in rats attenuated left ventricular remodeling and deterioration of performance after three months and prolonged survival after one year (Pfeffer JM et al.,1985; Pfeffer MA et al., 1985). Subsequently, the effect was also demonstrated in rats with enalapril (Sweet et al., 1987; Sweet et al., 1988; Hodsman et al., 1988b), and perindopril (Michel et al., 1988). These studies have prompted clinical studies which will be discussed in the next chapter.

What is (are) the underlying mechanism(s) responsible for these beneficial effects of captopril in the natural history of congestive heart failure due to hypertension and myocardial infarction? There is no doubt that the unloading of the ventricle by reduction of pre- and afterload is an important factor. However, this does not appear to be the whole story. Lack of a close parallel between blood pressure levels and the degree of cardiac hypertrophy in hypertension has been demonstrated both in the experimental and clinical situation (Sen et al., 1987). It has been suggested that antiadrenergic agents such as methyldopa or propranolol facilitate regression of cardiac hypertrophy, whereas more specific vasodilators such as hydralazine do not (Dzau, 1988d). Furthermore, regression of cardiac hypertrophy produced by aorta stenosis in rats only occurred after captopril and not after nifedipine or hydralazine, despite an equal blood pressure lowering effect which was attributed to either the local or systemic reduction of angiotensin II (Linz et al., 1988). Inhibition of the cardiovascular renin-angiotensin system and subsequent reduction of sympathetic nervous system activity may be important contributing factors. The same applies to myocardial infarction: local generation of angiotensin II and sympathetic activation may be essential in the process of remodeling. Inhibition of these neurohumoral systems may explain in part why converting enzyme inhibitors seem so effective in this situation. Therefore, the beneficial effects of captopril on remodeling and subsequent development of congestive heart failure, may be related to other factors than reduction of pre- and afterload.

2. CORONARY BLOOD FLOW

Local or systemic angiotensin II may influence coronary vascular tone and thereby coronary blood flow (Dzau, 1988). However, the effect of converting-enzyme inhibitors on coronary vascular resistance is usually masked by their effect on pre- and afterload during systemic administration. Coronary blood flow is determined primarily by the metabolic requirements of the myocardium and regulated to a major extent by autonomic nervous system mechanisms (Young, 1987). When myocardial oxygen demand is constant and changes in perfusion pressure occur the heart is able to regulate its own blood sup-

ply (autoregulation), which is the result of a concerted interaction of several mechanisms (Dole, 1987). Therefore, in vivo activation of angiotensin II or inhibition by angiotensin converting enzyme inhibitors may be counteracted by other mechanisms. More information about these effects can be obtained from experiments in the isolated rat heart, which showed some remarkable results.

First of all it was demonstrated that both angiotensin I and angiotensin II were able to induce vasoconstriction, corroborating both the vasoconstrictive properties of angiotensin II and the presence of a local converting enzyme in the endothelium of the coronary vasculature (Xiang et al., 1985). Second, when a converting enzyme inhibitor was added to the perfusate, coronary flow increased and the effect of angiotensin I was antagonized (Xiang et al., 1985; Linz et al., 1986). Third, pretreatment in vivo, both with captopril and other angiotensin converting enzyme inhibitors, resulted in a flow increase in vitro which persisted throughout the entire experiment (Xiang et al., 1985; Linz et al., 1986; van Gilst et al., 1988). Following captopril and zofenopril administration this effect was only significant when the rats were pretreated for 48 hours, and not when pretreatment lasted only one hour (van Gilst et al., 1988). This suggests that the presence of sufficient tissue concentrations of both compounds is necessary for their coronary vasodilating effects, however more evidence is needed for confirmation (van Gilst et al., 1988).

Although these experiments clearly showed that angiotensin converting enzyme inhibitors were able to improve coronary flow, the underlying mechanism proved puzzling. Although local inhibition of angiotensin II may be of primary importance in vivo, this was more difficult to understand in vitro as angiotensin II was below the detection limit in the coronary effluent in control hearts. Subsequently, attention was focused on the importance of the bradykinin potentiation by the angiotensin converting enzyme inhibitors, which can stimulate the release of both prostaglandin I2 and endothelium-derived relaxing factor (EDRF, see previous chapter). However, differential influences of angiotensin converting enzyme inhibitors turned out to be present. It was shown that captopril and zofenopril had a much more rapid and pronounced effect on the coronary flow than other angiotensin converting enzyme inhibitors (van Gilst et al., 1987; Appendix 8; van Gilst et al., 1988). As was discussed earlier, the sulfhydryl group could be responsible for this additional angiotensin converting enzyme-independent vasodilatory effect. Since both EDRF (identical to the nitric oxide radical) and sulfhydryl groups are involved in the mechanism of action of nitrates, this may also explain why captopril can potentiate the effects of isosorbide dinitrate (Appendix 8).

A more complex picture emerges from the in vivo animal experiments. When captopril was given either intravenously or intracoronarily to anesthetized dogs under normoxic conditions, it did not produce a marked change in coronary blood flow (Noguchi et al., 1985; Noguchi et al., 1987), implying that

the renin-angiotensin system plays only a minor, if any, part in the regulation of coronary hemodynamics. However, the degree of participation changes during myocardial ischemia or other conditions where the renin-angiotensin system is stimulated (Ertl et al., 1983; Liang et al., 1978; Drexler et al., 1985; Gavras and Liang, 1976; Drexler et al., 1987). It has been shown that some vascular beds are more sensitive to angiotensin II than others, especially the renal and coronary beds (Noguchi et al., 1987). Conversely, inhibition of the angiotensin converting enzyme with teprotide or captopril in the situation of an activated renin- angiotensin system, may induce increase of coronary blood flow, whereas blood flow decreases in other tissues, especially the liver and the splanchnic system (Gavras and Liang, 1976; Drexler et al., 1987).

This may be of special importance in ischemic heart disease. Several studies have shown that myocardial ischemia induces release of renin (Ertl et al., 1981; Liang et al., 1982; Ertl et al., 1985). Consequently, saralasin and captopril acted as coronary vasodilators in various models of myocardial ischemia (Ertl, 1987). However, this increase of myocardial blood flow was not seen by all investigators, presumably due to metabolic autoregulation and drop in coronary perfusion pressure (Liang et al., 1982; Berdaux et al., 1987). However, when systemic vasodilatory effects were excluded, saralasin completely prevented the further fall of coronary flow reduction during atrial pacing. This supports the hypothesis that a coronary constrictive tone mediated by angiotensin II exists in the presence of myocardial ischemia (Ertl et al., 1985).

Reduction of the systemic renin-angiotensin system appears to play an important role in the effect of captopril in myocardial ischemia, since nephrectomy significantly reduced its vasodilatory effect. However, a discrepancy was found between the rather small increase in plasma renin activity and the marked coronary vasodilatory effect after captopril was administered. Inhibition of a regional coronary vascular renin-angiotensin system, activated by myocardial ischemia, was proposed as an underlying mechanism (Ertl et al., 1985). Support was obtained by the finding that coronary extraction of renin occurs during experimental coronary occlusion (Ertl et al., 1985). As the experiments in the isolated rat heart indicate, converting enzyme inhibition may favourably alter the balance between vasoconstrictory and vasodilatory mechanisms with a reduction of angiotensin II on the one hand and a bradykinin-mediated stimulation of the release of prostaglandin I2 and EDRF on the other. Whether the sulfhydryl group also has additional effects in vivo, remains to be established.

The final question remains whether or not this increase in coronary flow can beneficially influence the course of events during ischemia and reperfusion. The answer appears to be affirmative. An important role in the cardioprotective effect of angiotensin converting enzyme inhibitors against reperfusion arrhythmias in vitro has been ascribed to improvement of regional perfusion

(Linz et al., 1986). In vivo the same applies to the reduction of the extent of infarction along the lateral edge of the zone of necrosis after 6 hours of experimental coronary occlusion (Ertl et al., 1982). Furthermore, abolition of the deterioration of myocardial function during moycardial ischemia, induced by coronary hypoperfusion and atrial pacing, has been shown (Ertl et al., 1987). Therefore, angiotensin converting enzyme inhibitors appear to be of value in ischemic heart disease, especially when the renin-angiotensin system is activated. A beneficial role may be postulated in patients during stable and unstable angina pectoris, whether or not in combination with congestive heart failure and hypertension, or when nitrate therapy is co-administered. The truthfulness of this hypothesis will be discussed in the next chapter.

3. ISCHEMIA/REPERFUSION-INDUCED MYOCARDIAL INJURY

As mentioned above, the renin-angiotensin system is activated during coronary artery occlusion. The essential question is whether this activation is beneficial for the patient or whether overshoot occurs. It has been suggested that the increase of renin synthesis by the kidney is of benefit to the kidney itself, but detrimental to the heart (Packer et al., 1987b). Angiotensin II is known to be a positive inotropic agent as well as a coronary and systemic vasoconstrictor (Koch-Weser, 1964). Increased angiotensin II production would further compromise the ischemic myocardium by reducing myocardial oxygen supply while increasing myocardial oxygen consumption due to both the enhanced inotropic state and the increased afterload. Furthermore, angiotensin II has been shown to produce coronary vascular lesions and endothelial damage, as well as myocardial infarction (Gavras et al, 1975). In addition, angiotensin II potentiates sympathetic nervous system activity, which further enhances myocardial oxygen consumption and increases myocardial damage (Karlsberg et al., 1981). If activation of angiotensin II is detrimental to the heart, it is tempting to suggest that inhibition of this peptide may be beneficial to the heart.

The latter concept led to a number of animal studies with converting enzyme inhibitors on ventricular performance, regional myocardial blood flow and infarct size after coronary artery occlusion. All studies, irrespective which angiotensin converting enzyme inhibitor was applied, showed an improvement of global ventricular performance when left ventricular failure was present, due to reduction of pre- and afterload (Liang et al., 1978; Sweet et al., 1984; Drexler et al., 1987; Shionoiri et al., 1987). The effect on regional myocardial blood flow has been discussed earlier. Finally, the effect on infarct size was studied.

In the first study which was published on this subject, a significant increase of collateral blood flow and reductions of both the myocardial area at risk and infarct size was observed with captopril treatment following 6 hours of coro-

nary occlusion in anesthetized dogs (Ertl et al., 1982). However, two subsequent studies with captopril, also in dogs, showed no limitation of infarct size 24 hours after coronary artery occlusion (Liang et al., 1982; Daniell et al., 1984). A conclusive explanation found for these negative results could not be found, although differences in anesthesia and duration of ischemia were postulated to be of importance. Further evidence of a beneficial effect of angiotensin converting enzyme inhibitors in the setting of acute myocardial infarction was obtained by the group of Lefer, who found reduction in infarct size following acute left coronary artery ligation after 48 hours in the rat and after 5 hours in the cat (Lefer and Peck, 1984; Hock et al., 1985). At the time of publication of these experiments, the observed effects were primarily attributed to a reduction of systemic angiotensin II, although kinin-mediated mechanisms were also suggested to be involved (Hock et al., 1985). However, when increasing evidence for the existence of an independent local renin- angiotensin system in the heart was presented, it was postulated that direct effects on the heart may also be involved. This idea was reinforced after it was demonstrated for the first time that captopril was able to reduce myocardial ATP breakdown and reperfusion arrhythmias after reversible ligation of the left coronary artery in the isolated rat heart (van Gilst et al., 1984). Clearly these effects were independent of its pre- and afterload reducing properties, and the results were subsequently corroborated with both captopril and other angiotensin converting enzyme inhibitors by the same, and other investigators (Appendices 3 and 4; Linz et al., 1986; Linz et al., 1987; Appendix 6; Li and Chen, 1987; Rochette et al., 1987; van Gilst et al., 1988).

Initially it was thought that local inhibition of angiotensin II formation was primarily responsible for these effects. This was supported by the finding that addition of angiotensin II to the perfusate strongly enhanced the occurrence of ventricular fibrillation (Linz et al., 1987). Apparently, this inhibition of angiotensin II was mediated by cardiac angiotensin converting enzyme inhibition, since the beneficial effect is limited to the active, converting enzyme inhibiting form (Appendix 4). However, no angiotensin II could be detected in the coronary effluent of control hearts (Appendix 3). When it was subsequently shown that concurrent administration of indomethacin abolished the beneficial effects of captopril, it was suggested that facilitation of prostaglandin synthesis, mediated by potentiation of bradykinin, appeared to play an important role (Appendix 4). The importance of bradykinin was further determined by experiments, which demonstrated on the one hand a marked reduction of the duration of ventricular fibrillation when bradykinin was added to the perfusate, and a complete negation of the beneficial effects of ramipril when a bradykinin antagonist was coadministered on the other (Linz et al., 1986; Schölkens et al., 1988). Therefore, both increased bradykinin and decreased angiotensin II levels seem to be crucial in preventing reperfusion damage.

An important question remains whether or not the effects of captopril in the isolated rat heart only occur when reperfusion is achieved. The same effects were obtained when captopril was given just before reperfusion, however to a lesser extent (Appendix 6). When a high dose was given (80 microg/ml), a significant improvement of the pressure-rate index was already observed during ischemia (Appendix 3). Studies with nuclear magnetic resonance demonstrated that pretreatment with capropril significantly reduced accumulation of inorganic phosphate at the end of ischemia, with AMP and ATP readily available for rephosphorylation to ATP upon reperfusion (Rahusen et al., 1988). In ramipril pretreated hearts myocardial lactate was significantly reduced and energy-rich phosphates significantly increased not only at the end of of the ischemic period, but also at the end of the control period, although to a lesser extent (Linz et al., 1987). Therefore, the protective effects start early, even before ischemia, and become increasingly manifest during the course of events, especially reperfusion.

These beneficial effects on reperfusion damage, which were already present at therapeutic plasma levels (Appendix 3), clearly needed confirmation in vivo. It was no surprise when subsequent experiments in the rat (Hock et al., 1985), the pig (Appendices 5 and 6) and the dog (Elfallah and Ogilvie, 1985; Westlin and Mullane, 1988) showed similar results in vivo. Pretreatment with a converting enzyme inhibitor reduced enzymatic infarct size (Hock et al., 1985; Appendices 5 and 6), lowered incidence of ventricular fibrillation upon reperfusion (Elfallah and Ogilvie, 1984; Westlin and Mullane, 1988) and improved segmental function upon reperfusion (Westlin and Mullane, 1988). The reduction of overflow of noradrenaline after reperfusion demonstrated strong similarities with the in vitro experiments (Appendix 5). Although differential effects appeared to be present between the various angiotensin converting enzyme inhibitors, all showed at least some effects. The differences appeared to be mainly due to alterations in duration of ischemia, which varied from 10 to 60 minutes. This influences the extent of myocardial damage and type of reperfusion arrhythmia (Manning and Hearse, 1984). In addition, as has been discussed in the previous chapter, some of the effects of captopril and other sulfhydryl-containing angiotensin converting enzyme inhibitors may be due to the capacity to scavenge oxygen-derived free radicals (Westlin and Mullane, 1988). Further comparative studies with other converting enzyme inhibitors without a sulfhydryl group will be necessary to determine the relative importance of this property.

Although questions remain about the dose needed, the underlying mechanism and the optimal time to start therapy, all these results appear to be very promising. Apparently, captopril could be of benefit in the setting of acute myocardial infarction, especially when reperfusion is due to occur. Clinical experience had been limited so far to patients with acute myocardial infarction

and left ventricular failure. With the increasing use of thrombolytic therapy, more attention is being focused on reperfusion-induced myocardial injury. Therefore, we thought it justified to initiate a clinical study with captopril in patients with acute myocardial infarction undergoing thrombolysis. The results will be discussed in the next chapter.

4. VENTRICULAR ARRHYTHMIAS

Life-threatening ventricular arrhythmias may be present in a variety of both acute and chronic conditions in which cardiac function is compromised. However, several mechanisms may be present in various circumstances. The extent to which the renin-angiotensin system is involved in the genesis of arrhythmias is largely unknown, although a contributory role can be postulated through stimulation of sympathetic nervous system activity.

Animal models of coronary artery occlusion and reperfusion can be used for understanding on this subject. In vitro, in the Langendorff perfused isolated rat heart, premature ventricular beats occur infrequently during ischemia, but upon reperfusion ventricular fibrillation occurs consistently. In vivo, in various animal models, there is a high incidence of ventricular tachycardia and/or ventricular fibrillation both during ischemia and reperfusion (Elfallah and Ogilvie, 1985). Our closed-chest pig model appears to be particularly useful because it allows us to study the inducibility of ventricular tachycardia during the chronic phase of myocardial infarction (Kingma et al., 1986; van Gilst et al., 1987; Appendix 6; de Langen et al., 1989). In the pig heart there is no substantial collateral blood flow that of man.

Experiments with captopril and other converting enzyme inhibitors in these models have shown remarkable results. In the isolated rat heart a high concentration of captopril completely prevented ventricular fibrillation upon reperfusion (van Gilst et al., 1984; Appendices 3 and 4) Even at low concentrations a significant reduction of the incidence and duration of ventricular fibrillation was revealed (Appendix 3). This was also shown for other angiotensin converting enzyme inhibitors such as ramiprilat (Linz et al., 1986) and perindopril (Rochette et al., 1987), even when given just prior to reperfusion (Linz et al., 1987). Results with enalaprilat were more ambiguous, but this may have been related to the dose used (Appendix 4; Li and Chen, 1987).

The underlying mechanisms of this antiarrhythmic effect appear to be closely related to the reduction of reperfusion damage, which has been discussed earlier. Captopril is not an antiarrhythmic agent in the sense that it has a direct action on ionic channels in the cell membrane (unpublished observation). Reduction of noradrenaline overflow, which has been described both after captopril and ramiprilat (Appendices 3 and 4), appears to be of major importance,

although other mechanisms such as the improved metabolic status upon reperfusion, the increase of myocardial high energy phosphate content and the reduction in lactate concentration may also be involved. Potentiation of bradykinin in general and of bradykinin mediated enhancement of prostaglandin synthesis in particular may explain these effects of converting enzyme inhibitors. On the one hand, addition of prostaglandin I2 or bradykinin to the perfusate prevented ventricular fibrillation upon reperfusion in control hearts and potentiated the antiarrhythmic effects of converting enzyme inhibitors (Appendix 4; Li and Chen, 1987), while on the other addition of indomethacin or a selective bradykinin antagonist to the perfusate completely abolished these effects (Linz et al., 1987, Schölkens et al., 1988b). Inhibition of the synthesis of locally generated angiotensin II may be another reason, but this could not be demonstrated in vitro.

In vivo, when the renin-angiotensin system is intact, an important contributory role of angiotensin II, both locally and systemically, to arrhythmogenesis appears to be much more likely. Reduction of coronary blood flow, facilitation of sympathetic transmission, enhanced systolic wall stress due to increased afterload and potassium loss by stimulation of aldosterone, and local shortening of ventricular refractoriness all promote electrical instability. Therefore, inhibition of angiotensin II synthesis by converting enzyme inhibitors may lead to a reduction of ventricular arrhythmias during both ischemia and reperfusion. This has been described following coronary occlusion in the rat with captopril and perindopril (Rochette et al., 1987), in the pig with captopril (Coker and McGrafth, 1985), and in the dog with both captopril and enalapril (Elfallah and Ogilvie, 1985). This may be primarily attributed to reduction of cardiac overload due to its vasodilatory effect since this effect appears to be similar to other afterload-reducing agents (Elfallah and Ogilvie, 1985). However, when blood pressure decreases excessively following ischemia, this beneficial effect will no longer be present due to a critical reduction of coronary perfusion (Elfallah and Ogilvie, 1985). This may explain why significant reduction of ventricular tachycardia and ventricular fibrillation was not seen during ischemia following captopril pretreatment in the closed chest pig model (Appendices 5 and 6).

Similar to the experiments in vitro, reduction of ventricular fibrillation has been described in the dog upon reperfusion after shortlasting ischaemia (15-30 minutes) following pretreatment with enalaprilat (Westlin and Mullane, 1988; Elfallah and Ogilvie, 1985) and captopril (Westlin and Mullane, 1988). No ventricular fibrillation was seen in our closed chest pig model, which was probably related to the prolonged period of myocardial ischemia (60 min). An accelerated idioventricular rhythm (AIVR) occurred in almost all pigs without significant differences between captopril treated and untreated hearts. AIVR is a generally benign ventricular arrhythmia and a marker for both myocardial

necrosis and reperfusion of the infarct-related vessel (Cercek et al., 1987). This arrhythmia is known to be caused by abnormal automaticity from the His-Purkinje system. No relation with infarct size exists (Gorgels et al., 1988).

Interestingly, in captopril pretreated animals, a significant reduction of myocardial noradrenaline overflow was seen, similar to the in vitro studies, but apparently this did not result in reduction of AIVR (Appendices 5 and 6). However, this interference of captopril with local release of noradrenaline may be very important with regards to arrhythmogenesis late after myocardial infarction. Infusion with angiotensin II 2 weeks after myocardial infarction in the pig causes inducible ventricular tachycardia, either sustained or non-sustained, in 5 out 9 non-inducible pigs (de Langen et al., 1989). These arrhythmias in the chronic phase of myocardial infarction are known to be caused by intraventricular reentry. This was associated with a shortening of ventricular refractoriness, probably mediated by facilitation of noradrenergic transmission in the heart. Administration of captopril either intravenously or following oral pretreatment significantly reduced inhibition of inducible ventricular tachycardia (Kingma et al., 1986; Appendix 6; de Langen et al., 1988), again showing that modulation of the renin-angiotensin system after acute myocardial infarction has a beneficial effect on the development of malignant ventricular arrhythmias.

One may speculate whether the earlier described beneficial effects of captopril on ventricular enlargement during the chronic phase (remodeling) (Pfeffer JM et al., 1985), will further contribute to this beneficial effect. Improved hemodynamic function, increased left ventricular mass/volume ratio and decreased systolic wall stress will reduce oxygen consumption of the damaged myocardium, possibly leading to a reduction of ventricular rhythm disturbances. However, no data are yet available although the prolonged survival, which has been described following captopril treatment in rats after myocardial infarction (Pfeffer MA et al., 1985), may be at least partly due to this arrhythmia-preventing effect.

In summary, early treatment during the course of ischemic heart disease may prevent the development of serious ventricular arrhythmias occurring both early and late after myocardial infarction. This clearly warrants further studies in the clinical situation in man, as will be discussed in the next chapter.

CHAPTER IV

EFFECTS OF CAPTOPRIL IN PATIENTS

1. CONGESTIVE HEART FAILURE

In chapter I captopril was concluded to be an effective oral agent for the management of heart failure resistant to digitalis and diuretic therapy. Early studies with captopril conducted in patients with advanced heart failure clearly indicated a sustained hemodynamic benefit during long-term captopril therapy with continued reduction in left ventricular filling pressure and increase in cardiac and stroke output (Ader et al., 1980; Awan et al., 1982; Kramer et al., 1983). This is also shown with other angiotensin converting enzyme inhibitors, such as ramipril (Appendices 1 and 2). Although a significant reduction in blood pressure after single doses of captopril was a consistent finding, blood pressure usually returned to near baseline levels with continued therapy (Romankiewicz et al., 1983, Appendix 2).

Initially, attention was primarily concentrated on the hemodynamic effects. This changed when it became increasingly clear that there was a lack of relationship between the hemodynamic effects of captopril and subsequent clinical response (Levine et al., 1980; Fouad et al., 1982; Massie et al., 1984). Furthermore, a complex and variable relationship appeared to exist between the early and late hemodynamic effects in patients with severe heart failure (Packer et al., 1983). The clinical efficacy of captopril in heart failure was definitely established by two placebo-controlled trials which demonstrated a significant improvement in the New York Heart Association functional class value and increase in exercise tolerance with a follow-up as long as two years (Captopril Multicenter Research Group, 1983; Captopril Multicenter Research Group I, 1985). Comparative studies with other classes of vasodilating agents, such as prazosin (Bayliss et al., 1985; Mettauer et al., 1986; Bayliss et al., 1986) and nifedipine (Agostini et al., 1986), showed superior effects with regard to functional capacity. Unfortunately, little information is available comparing the angiotensin converting enzyme inhibitors with nitrate therapy, for which there is the greatest evidence of clinical efficacy. One study demonstrated an enhancement of cardiac sympathetic tone by the combination of hydralazine and isosorbide dinitrate but not after captopril (Daly et al., 1986).

The reason why captopril and other angiotensin converting enzyme inhibitors are advantageous in contrast to other vasodilating agents is not quite clear. The effect may be attributed due to the attenuation of certain neuroendocrine control systems, such as the renin-angiotensin system and the sympathetic nervous system, which are stimulated by other vasodilating agents (Met-

tauer et al., 1986, Lipkin and Poole-Wilson, 1985; Chatterjee et al., 1982). Another potential reason for the differences in clinical effects between angiotensin converting enzyme inhibitors and other vasodilating agents may be due to different patterns of regional blood flow. Many of the manifestations of low-output congestive heart failure are secondary to reduced blood flow to various organs and regions of the body (Levine, 1985; Leier, 1988). Most important with regards to exercise testing are the effects on blood flow to skeletal muscle and metabolism. Whereas acute converting enzyme inhibition usually exerts neither substantial improvement in exercise hemodynamics nor interferes with leg performance during exercise (Timmis et al., 1987), skeletal muscle blood flow and oxygen uptake gradually increase with long-term angiotensin converting enzyme therapy (Mancini et al., 1987; Drexler et al., 1988). This appears primarily due to inhibition of tissue angiotensin converting enzyme and favorably discriminates captopril from other vasodilating agents.

The main reason why captopril and other converting enzyme inhibitors have gained in popularity only slowly was the fear for serious side-effects. Adverse effects of captopril fall into two categories - those assumed to be related to the sulfhydryl group (skin rashes, alteration in taste perception, neutropenia and proteinuria) and those associated with converting enzyme inhibition (hypotension, renal insufficiency, hyperkalemia, cough and angioedema) (Gavras and Gavras, 1988a). Adverse reactions associated with the sulfhydryl group were especially prominent in patients on unnecessarily high doses of captopril and are much less frequent at lower doses (Editorial, 1988). Removal of the sulfhydryl group per se may confer no substantial benefit. The most important side-effects of angiotensin converting enzyme inhibitors in patients with congestive heart failure are hypotension and renal dysfunction. Although severe hypotension is often well tolerated (Appendix 1), starting doses should be as low as possible (Hodsman et al., 1983; Cleland et al., 1985b; Appendices 1 and 2) . The occurrence of renal insufficiency is the result of loss of angiotensin II-mediated systemic and intrarenal vasoconstrictive effects. These are needed to maintain renal perfusion pressure and glomerular filtration rate in low-output states (Packer et al., 1987a). Although initial decrease of renal function may occur, recovery and improvement is often seen during the subsequent treatment period (Mujais et al., 1984). However, when high doses of a long-acting angiotensin converting enzyme-inhibitor, such as enalapril, are given, this may induce prolonged hypotensive effects that even may compromise cerebral and renal function (Packer et al., 1986). Therefore, the dose should be titrated to the patient's needs and not on the basis of maximal hemodynamic effects (Appendix 2). Preliminary results with lisinopril, another long-acting angiotensin converting enzyme inhibitor, using a titrated dosage scheme even suggest an improvement of efficacy, but this awaits further confirmation (Powers et al., 1987).

Now that the place of angiotensin converting enzyme inhibitors in the treatment of moderate to severe heart failure is firmly established, attention is concentrated on the efficacy in early, less severe heart failure. In a large multicenter, double-blind placebo-controlled study in patients with mild to moderate heart failure, captopril was convincingly shown to be a more effective alternative for digoxine, leading to a significant increase in exercise capacity and reduction of ventricular arrhythmias (Captopril-Digoxin Multicenter Research Group, 1988). However, compared to diuretic therapy, captopril appeared to be less effective, especially when signs of fluid retention had been present previously (Cowley et al., 1986; Richardson et al., 1987). Therefore, it seems appropriate to advise early treatment with a diuretic first and then an angiotensin converting enzyme inhibitor, although this may vary depending on the signs and symptoms of the patient. Perhaps, it would be more advisable to commence the combination immediately. Only following further progression should a positive inotropic agent be indicated.

The unanswered question remains whether captopril and other converting enzyme inhibitors should be indicated in the symptomless patient with cardiac dysfunction due to idiopathic cardiomyopathy, ischemic heart disease or hypertension. It has been repeatedly demonstrated that similar to animal experiments angiotensin converting enzyme inhibition leads to regression of left ventricular hypertrophy in patients with hypertension (Dunn et al., 1984; Nikashima et al., 1984; Ventura et al., 1985). In addition, two important studies have revealed that early treatment with captopril in symptomless patients with cardiac dysfunction following acute myocardial infarction attenuate the development of ventricular dilation, identical to the earlier described effect in infarcted rats (Sharpe et al., 1988; Pfeffer et al., 1988). These studies are strongly in favor of the hypothesis that early treatment with captopril and other angiotensin converting enzyme inhibitors may inhibit or slow down the progression to overt heart failure. The proof of this hypothesis must await the completion of currently conducted clinical trials. However, theoretically one might expect this hypothesis to be true, when the beneficial effects of angiotensin converting enzyme inhibition on the heart's economy are taken into account (Gavras, 1988b).

2. CORONARY BLOOD FLOW/ANGINA PECTORIS

Fear for deterioration of their clinical status due to an excessive drop in blood pressure has brought clinical investigators to exclude patients with angina pectoris from almost all studies with captopril and other angiotensin converting enzyme inhibitors in hypertension and congestive heart failure. Although ischemic heart disease is common in this population, it is remarkable

that only rarely angina pectoris or myocardial infarction have been described as a side effect, despite the sometimes pronounced decrease in blood pressure (Massie, 1988). Therefore, angiotensin converting enzyme inhibitors are perhaps less dangerous in patients with angina pectoris than initially thought, which leads to the question whether they may also be effective in alleviating the symptoms.

There are several reasons to assume a beneficial effect, as has already been discussed in the previous chapter. Angina pectoris is the result of a disbalance between myocardial oxygen supply and consumption. The balance may be restored after administration of an angiotensin converting enzyme inhibitor. For instance, oxygen consumption will decrease due to the reduction of the pressure-rate index and perhaps wall-stress, while at the same time coronary vasodilation may occur, as the animal experiments have shown (see previous chapter). These effects may be present both on a local, intracardiac and on a systemic, circulatory level. Clinical evidence sofar suggests that these theoretical and experimental considerations may also be relevant in man, although variable results have been described.

Most attention has focused on the effects of captopril and other angiotensin converting enzyme inhibitors on coronary blood flow. The first studies on this subject described the acute effects of captopril and teprotide on coronary hemodynamics in congestive heart failure (Chatterjee et al., 1982; Rouleau et al., 1982; Halperin et al., 1982; Powers et al., 1982; Faxon et al., 1984). Consistently, a reduction in coronary blood flow was seen, corresponding to a reduction of myocardial oxygen consumption. However, criteria for myocardial ischemia such as coronary sinus oxygen saturation and lactate extraction remained unchanged, although in individual patients myocardial lactate production was observed (Rouleau et al., 1982; Chatterjee et al., 1982). The decrease in myocardial oxygen consumption appeared to be primarily due to a reduced pressure-rate product, leading to a lower metabolic requirement of the myocardium. Interestingly, this correlation was not present with prazosin (Rouleau et al., 1982) suggesting that other factors than the afterload-reducing properties may influence the effects of vasodilators on the coronary circulation.

These different effects of captopril were also shown by investigators in patients without congestive heart failure. An increase in coronary blood flow was found in healthy subjects (Faxon et al., 1982) and in patients with hypertension with no evidence of ischemic heart disease (Magrini et al., 1987), despite unchanged or even with a reduced pressure-rate index. Potentiation of bradykinin and subsequent stimulation of prostaglandin I2 may also contribute to these effects, as was demonstrated by an abolishment of the vasodilating effect of capopril by pretreatment with indomethacin (Mattioli et al., 1982). However, these vasodilatory effects were only shown in the presence of an activated renin-angiotensin system and were not found in another study with captopril

in patients with hypertension and angina pectoris (Daly et al., 1984). Following additional data suggesting a coronary vasodilating effect of angiotensin converting enzyme inhibitors (Dagianti et al., 1987; De Marco et al., 1987), more definite proof was obtained by the demonstration of coronary vasodilation without systemic effects after intracoronary administration of enalaprilat (Foult et al., 1988). The latter authors were the first to describe a negative effect of a converting enzyme inhibitor on left ventricular contractility despite improved hemodynamics. Decreased contractility and reduced neurohumoral activity may explain why myocardial oxygen consumption and pacing-induced ischemia in normotensive patients with coronary artery disease were significantly reduced by intravenous administration of enalaprilat (Remme et al., 1988).

As mentioned earlier, it is difficult to clarify underlying mechanisms of the effects of converting enzyme inhibitors in in vivo studies. It should however be emphasized that all the above- mentioned results were obtained after acute administration, where the effects on plasma renin angiotensin system tends to dominate the picture. Much less is known about the chronic effects of angiotensin converting enzyme inhibitors on the coronary circulation. This is especially important because inhibition of the cardiac renin-angiotensin system can, at least theoretically, enhance captopril's benficial effects on the balance between myocardial oxygen consumption and supply. As in congestive heart failure and hypertension, it may take weeks or months before an ultimate effect is established.

This may also apply to the potential therapeutic efficacy of these drugs in patients with angina pectoris. Limited data sofar have restricted its use to acute or short term treatment (up to 2 weeks). The first double-blind, placebo-controlled study by Daly et al. was published in 1985 and showed that a low dose of 12.5 or 25 mg captopril given once daily for two weeks resulted in a significant increase in exercise time (Daly et al., 1985). However, captopril was only given to patients with a systolic blood pressure above 120 mmHg and its therapeutic effect was limited to those patients in whom captopril decreased systolic arterial pressure more than 10 mmHg at rest and throughout the exercise test. Subsequent studies with a single dose of captopril in normotensive patients also demonstrated this association between improvement of exercise testing results and the reduction of the pressure-rate index (Strozzi et al., 1987; Keck et al., 1987). Interestingly, afterload reduction was only mild and it was suggested that the reduction in preload may be important as well (Keck et al., 1987). This may also explain why only captopril, but not alpha-methyldopa improved exercise time in patients with hypertension and ischemic heart disease, despite equal blood pressure lowering effects (Strozzi et al., 1985). Accordingly, a significant improvement in ST-segment depression and decrease in the percentage of ischemic area during exercise without significant changes in

the pressure-rate index was described in patients with coronary insufficiency following 48 hours of treatment with captopril (Tardieu et al., 1986).

Similar results have been obtained with enalapril (Strozzi et al., 1988) and quinapril (Bussman et al., 1988a). Yet unanswered is whether the presence of a sulfhydryl group yields additional advantages for captopril, both with regard to its vasodilating properties and to a potential interaction with nitrate therapy. In clinical practice captopril and nitrate therapy will often be given combined and an interaction may have important consequences. Our clinical study in patients with stable angina pectoris points in this direction (Appendix 9), but obviously comparative studies with other angiotensin converting enzyme inhibitors without a sulfhydryl group are required.

A study with enalapril in patients with proven coronary heart disease further shows the importance of long term treatment (Rietbrock et al., 1988). Although a significant reduction of exercise-induced ST-segment depression was already present after the first dose, this effect was further enhanced after two weeks treatment, despite similar, mild effects on blood pressure and heart rate during exercise. Remarkably, the authors of this study suggested that dilatation of the coronary arteries by stimulation of EDRF may contribute to this effect (Rietbrock et al., 1988), again suggesting that local factors may play an increasingly important role during the course of the treatment.

At this point, a word of caution is warranted. It should be emphasized that most studies were carried out in small groups of patients with a deficient design or exercise testing protocol. Studies showing no benefit of angiotensin converting enzyme inhibitors in patients with angina pectoris have also been reported (Hauf et al., 1987; Rosenthal et al., 1987; Abrams and LeTourneau, 1987). With regard to safety, marked hypotension may occur, especially when the renin-angiotensin system is activated, and this can exceed the beneficial effects of decreased myocardial oxygen consumption, as well as any of the beneficial effects on the coronary vasculature. Hypotension may explain why worsening of exercise time following captopril was found in a recent placebo controlled study in patients with heart failure and angina pectoris (Cleland et al., 1988). This drop in blood pressure may also occur in normotensive patients, especially when higher doses are used (Bussman and Goerke, 1988b). Therefore, dose titration by captopril and other angiotensin converting enzyme inhibitors should be performed carefully, starting with a low dose and increasing the dose only after long intervals. Finally, a coronary steal effect has been described in a study by Tardieu et al. in 1986, but needs further investigation.

Obviously, the treatment of angina pectoris with angiotensin converting enzyme inhibitors in general and captopril in particular is not yet clearly defined. Additional clinical data are needed to define the type of patient which may benefit, the type of angiotensin converting enzyme inhibitor (sulfhydryl group),

the interaction with nitrate therapy and the dosage. Initial results are promising and further studies are in progress.

3. ACUTE MYOCARDIAL INFARCTION

Coronary artery occlusion sets into motion a complex series of local and systemic processes which initially will determine the survival of the area of myocardium at risk and ultimately the survival of the individual. The hemodynamic significance of a coronary artery occlusion depends upon a series of factors, of which the most important are the area at risk, the presence and function of collateral flow, and the functional state of the residual non-ischemic part of the left ventricle (Cobbe, 1987). The severity of the initial disturbance in global left ventricular function will determine the overall neurohumoral response. Patients with large areas of infarction tend to develop cardiogenic shock, late ventricular arrhythmias and/or ventricular fibrillation. These are all associated with a poor prognosis. The question which arises is whether the neurohumoral response seen after acute myocardial infarction can be deleterious in the development of these life- threatening complications. If so, inhibition can have beneficial effects.

It has been repeatedly shown that catecholamines are released acutely following myocardial infarction in relation to the extent of left ventricular damage (Jewitt et al., 1969; Videbaek et al., 1972; Benedict et al., 1979; Karlsberg et al., 1981; Bertel et al., 1982; McAlpine et al., 1987). More recently, observations indicate that the renin-angiotensin system can also be activated, although more slowly with a maximal activation after three days (Michorowski and Ceremuzynski, 1983; Vaney et al., 1986; McAlpine et al., 1988). High concentrations of both catecholamines and angiotensin II are frequently associated with the presence of heart failure, shock or ventricular arrhythmias (Jewitt et al., 1969; Karlsberg et al., 1981; McAlpine et al., 1988). As has been explained in the previous pages, this activation of vasoconstrictor mechanisms may be detrimental due to a negative effect on the ratio between oxygen supply and demand, which influences the extent of myocardial infarction. This is further supported by studies with beta- adrenoceptor antagonists, which have shown a reduction in infarct size and an improvement of survival when given early after acute myocardial infarction (Yusuf et al., 1988). However, in the group of patients with the poorest prognosis, namely those patients in whom heart failure is developing, these drugs are contraindicated due to their negative inotropic properties.

In this setting captopril and other angiotensin converting enzyme inhibitors make an attractive alternative. Beneficial hemodynamic effects have been reported in patients with acute left ventricular failure secondary to myocardial

infarction following administration of captopril (Brivet et al., 1981; Bounhoure et al., 1982; Wenting et al., 1983; McAlpine et al., 1987). Captopril reduced both pre- and afterload and no reflex tachycardia occurred; sometimes heart rate even decreased. Clearly these effects result in a reduction of myocardial oxygen demand, beneficially influencing the extent of myocardial infarction. Since the renin-angiotensin system can also be activated in patients with acute myocardial infarction without heart failure (McAlpine et al., 1988), this may also apply to a much broader group of patients. Unfortunately, data of the effects of captopril and other angiotensin converting enzyme inhibitors in patients with uncomplicated acute myocardial infarction are almost completely lacking except for one abstract, which indeed described beneficial effects (Abdel-Aziz, 1987). The reason for this lack of data is without doubt the potential danger of a deleterious effect on coronary perfusion due to an excessive drop in systemic arterial pressure (Wenting et al., 1983).

It is remarkable however, that the administration of captopril in patients with left ventricular failure due to acute myocardial infarction has only seldomly resulted in the onset or worsening of angina or electrocardiographic abnormalities, despite the fact that a marked drop in blood pressure was sometimes seen. The reason for this remains to be established, but one may speculate whether local effects of captopril are responsible for the maintenance of an adequate perfusion pressure, as described earlier. However, it should be noted that in the clinical studies sofar, captopril was given at variable time intervals after the onset of myocardial infarction. When given in the acute ischemic phase, especially when therapy is combined with thrombolytic therapy, different effects may occur, necessitating a very careful dosing regime.

In the clinical situation captopril might primarily exert beneficial effects on the size of the myocardial infarction by altering the ratio between oxygen supply and consumption. As mentioned previously, this is not simply a matter of reduction of pre- and after load, but other, yet undetermined intracardiac mechanisms may play a role. In addition, as demonstrated by our animal experiments, captopril may have direct cardioprotective, i.e. tissue salvaging, effects leading to a reduction in reperfusion damage. These effects may also be relevant in the clinical situation because, with the increasing application of thrombolytic agents and coronary balloon angioplasty for emergency revascularization, early reperfusion has become a practical reality for large numbers of patients (O'Neill et al., 1988). Furthermore, it has been shown that even without thrombolytic therapy spontaneous reperfusion is not uncommon (DeWood et al., 1980; Ong et al., 1983). Despite the obvious benefits of reperfusion, many investigators have become increasingly concerned that early reperfusion may represent a "double-edged sword", i.e., that it paradoxically may sometimes cause additional myocardial injury (Braunwald and Kloner, 1986; van Gilst, 1986). Several mechanisms may be involved, among which calcium overload,

catecholamine efflux, generation of free radicals and probably also activation of angiotensin II. Therefore, there is a theoretical rationale for concomitant use of agents such as beta- adrenoceptor antagonists, nitrates, calcium channel blockers and free radical scavengers. This awaits extensive investigation. Captopril combines several, desired effects of these drugs as has been shown experimentally: it can scavenge free radicals, it blunts the catecholamine response, it can elicit coronary vasodilation and stimulate beneficial autocoïds such as prostacyclin and bradykinin.

For these reasons a clinical dose-finding study with captopril was performed in patients with acute myocardial infarction in whom early reperfusion was attempted by thrombolytic therapy (Appendix 7). Severe hypotension occurred following intravenous administration of captopril, possibly due to an interaction with streptokinase, but oral treatment was tolerated well. The limited data available on ventricular arrhytmias suggest that captopril may indeed beneficially affect the clinical outcome. In any case, blunting of the catecholamine response following administration of captopril can also occur in patients. Again, these observations suggest that experimental data can be extrapolated to the clinical situation.

Such is also the case with remodeling, the process of dilation of the left ventricle following myocardial infarction, as also described in rats. Echocardiographic studies have demonstrated that thinning and dilatation of the infarcted myocardium can occur in the clinical situation during the first few months after acute myocardial infarction (Eaton et al., 1979; Erlebacher et al., 1982). This may have important consequences for survival since infarct expansion may forebear myocardial rupture and aneurysm formation (Visser et al., 1986), and ventricular dilation is the most powerful predictor of mortality in patients after acute myocardial infarction (White et al., 1987).

Two separate studies have demonstrated that early treatment with captopril in asymptomatic patients with acute myocardial infarction and an ejection fraction of 45 percent or less, can beneficially influence this process of remodeling (Sharpe et al., 1988; Pfeffer et al., 1988). Similar to the data in the rat, an improvement of myocardial function was found after one year of treatment, leading to improved exercise tolerance. It is only reasonable to assume that eventually survival will also improve, as this was also shown in the rat. The ongoing S.A.V.E. study (Survival and Ventricular Enlargement) will hopefully ascertain whether this assumption is true.

In conclusion, captopril appears to be very promising as a cardioprotective agent in the early and late stages of myocardial infarction. It may be a good alternative for beta-adrenoceptor antagonists because these drugs are contraindicated in the patients with the poorest prognosis, namely the patients with symptomatic left ventricular dysfunction. However, more studies are needed with regard to efficacy and safety before this therapy can be advocated as a

prophylactic drug after acute myocardial infarction. For this reason we are currently conducting a double blind, placebo-controlled, multicenter study with captopril in patients with acute myocardial infarction, regardless of eligibility for thrombolytic therapy.

4. VENTRICULAR ARRHYTHMIAS

As mentioned before, captopril is not an antiarrhythmic in the sense that it alters the action potential of the myocardial cell. Despite this fact, it has been shown that captopril and other converting enzyme inhibitors can significantly reduce ventricular arrhythmias in patients with early and late stages of congestive heart failure (Cleland et al., 1984; Webster et al., 1985; Cleland et al., 1985a). Several mechanisms have been postulated.

Angiotensin converting enzyme inhibitors increase serum potassium by inhibition of aldosterone. Hypokalemia may directly predispose to the occurrence of ventricular arrhythmias in patients with severe left ventricular dysfunction, especially in the presence of concomitant therapy with digitalis (Francis, 1986; Cleland et al., 1987). It has been shown that reduced stores of potassium are often present in patients with severe heart failure, primarily due to diuretic therapy (Cleland et al., 1987). Correction may therefore beneficially influence these arrhythmias. The importance of this effect is further shown by our study in patients with moderate to severe heart failure, where no beneficial effects on ventricular arrhythmias were seen when serum potassium was kept in the normal range at the onset of the study (Appendix 2). However, arrhythmias in these patients are almost certainly multifactorial in origin and involve other mechanisms, such as increased wall tension, focal ischemia and increased neurohumoral activity (Francis, 1986). All these mechanisms can be influenced beneficially by angiotensin converting enzyme inhibition, although the contribution of each to the overall effect is difficult to assess. As disease progresses, it may become less significant because reserves are less and damage has become irreversible. Therefore, the greatest benefit with regard to arrhythmias can probably be derived from early treatment by attenuating the development of left ventricular dysfunction, a process closely related to arrhythmogenesis (Bigger, 1986). The same applies to hypertension. There is reason to believe that not hypertension itself but the subsequent development of left ventricular hypertrophy is the cause of malignant ventricular arrhythmias (Messerli et al., 1984; McLenachen et al., 1987; Bethge et al., 1987).

This is matter of some importance since malignant ventricular arrhythmias are the cause of death in many patients (Francis, 1986). The overall mortality for patients with congestive heart failure is quite considerable. Approximately 50% of those with NYHA classification III-IV will die within one year. Inde-

pendently of the severity of the disease, sudden death comprises approximately 40% of all causes of death in congestive heart failure (Massie and Conway, 1987; Bigger, 1987). In a series of several studies, on the average, 87% of patients with congestive heart failure had couplets and/or multiform ventricular premature beats. The incidence of non- sustained ventricular tachycardia varied from 25% to 80% of the patients (most commonly about 50%) (Francis, 1986). Although sudden death is not identical to death following malignant arrhythmias, the latter is most likely one of its major contributors (Dargie et al., 1987). Patients with frequent ventricular ectopic activity had a significantly less favourable long-term survival than patients with less frequent ventricular ectopic beats.

If angiotensin converting enzyme inhibitors indeed reduce the incidence and prevalence of ventricular arrhythmias, this should lead to an improvement in survival. The most convincing evidence sofar for a beneficial effect on mortality can be derived from the CONSENSUS study. This study demonstrated that enalapril, in patients with moderate to severe congestive heart failure, significantly reduced mortality (CONSENSUS Trial Study Group, 1987). Pooled analysis showed that this finding could also be extrapolated to captopril and other converting enzyme inhibitors (Furberg and Yusuf, 1988). However in the CONSENSUS study no influence on sudden death was observed. Furthermore, these beneficial effects on mortality were also described after administration of the combination of hydralazine and nitrate therapy (Veterans Administration Cooperative Study, 1986), suggesting that it is primarily the unloading of the heart that is responsible for the beneficial effect and not an antiarrhythmic effect per se. After all, left ventricular dysfunction and ventricular arrhythmias are independent predictors of subsequent mortality (Bigger, 1986). Therefore, clinical proof is lacking that the purported antiarrhythmic effects contribute significantly to the improvement in survival. Especially in patients with moderate to severe heart failure improvement of left ventricular dysfunction might be the primary underlying mechanism. However, this may be different in patients with less severe heart disease. A reduction in sudden death was suggested in a follow-up of a large, controlled study with captopril (Newman et al., 1988). Clearly this relationship between angiotensin converting enzyme inhibitors and reduction of ventricular arrhythmias on the one hand and reduction of ventricular arrhythmias and prolongation of survival on the other needs further studying before any definite conclusion can be drawn.

Special attention should be paid to the situation of acute myocardial infarction. Ventricular fibrillation is a major cause of death during acute occlusion of one or more coronary artery branches. Important factors that contribute to electrophysiological instability are among others increased cellular potassium outflow, catecholamine release, lactate production and the accumulation of free fatty acids (Weiss, 1987). Stimulation of the renin-angiotensin system will

also occur (McAlpine et al., 1987), which, as described before, can promote ventricular arrhythmias. Therefore, angiotensin converting enzyme inhibitors may be expected to exert a beneficial effect, but it is very doubtful whether in man this rise in angiotensin II levels is fast enough to be of any importance for the development of lethal ventricular arrhythmias during the first hours post myocardial infarction.

Thus, it seems likely that potentially beneficial effects on arrhythmogenesis develop gradually. This also appears to be the case when reperfusion occurs either spontaneously or following thrombolytic therapy. Similar to the experiments in the pig and in contrast to the in vitro rat heart experiments, initially only an accelerated idioventricular rhythm (AIVR) occurs, which is considered a relatively benign marker of reperfusion (Goldberg et al., 1983; Cercek et al., 1987; Gorgels et al., 1988). During the subsequent course, ventricular arrhythmias may occur due to intraventricular reentry. A similar response occurs in both reperfused and non-reperfused hearts, despite differences in morphology (McComb et al., 1988). Persistent high levels of noradrenaline and angiotensin II probably trigger these late arrhythmias, i.e. after 48 hours (McAlpine et al., 1988). Support is given by animal experiments, which showed a reduction of inducible sustained ventricular arrhythmias two weeks after myocardial infarction following administration of captopril. Since many studies have shown a statistically significant and reasonably strong association between ventricular arrhythmias recorded 7 to 21 days after acute myocardial infarction and death over subsequent years (Bigger, 1987), reduction by captopril and other angiotensin converting enzyme inhibitors may favourably alter the eventual outcome.

No controlled clinical investigations concerning the effects of captopril on ventricular arrhythmias following myocardial infarction are presently available. Only preliminary data give some indication of a beneficial effect (Abdez-Aziz, 1987; Toussaint and Bönner, 1988). We hope to obtain more information on this subject in our above-mentioned placebo-controlled study in patients with acute myocardial infarction by Holter monitoring.

CHAPTER V

SUMMARY AND CONCLUDING REMARKS

This thesis describes the experimental and clinical effects of captopril on the heart. Captopril was the first angiotensin converting enzyme inhibitor for oral administration. This enzyme is responsible for the conversion of angiotensin I in angiotensin II, one of the most potent pressor substances known, and the catabolism of bradykinin, a vasodilating substance. However, angiotensin converting enzyme inhibitors in general, and captopril in particular, are more than only vasodilating agents. Different effects may occur due to different mechanisms of action, which may result in different therapeutic actions in animal models and patients. This is demonstrated by our experimental and clinical data (Appendices 1-9) and supported by the literature (Chapters II-IV).

In Appendices 1 and 2 the effects of captopril were studied in patients with congestive heart failure. Inhibition of the angiotensin converting enzyme resulted in short- and long-term hemodynamic and symptomatic benefits. Captopril and longer acting converting enzyme inhibitors, such as ramipril, produce similar benefits, provided that the starting dose is low and further therapy is individualized. It remains to be proven whether or not these drugs have antiarrhyhmic properties when serum potassium concentration is normal.

Although the hemodynamic changes in our study correlated closely with changes in the circulating renin-angiotensin system, this was not always paralleled by symptomatic improvement. Local effects, such as changes in regional blood flow, may also be important for clinical efficacy. Essential for the understanding of these effects is the presence of local renin-angiotensin systems in tissues such as the blood vessel wall, the heart, the adrenal, and the brain. Local inhibition by angiotensin converting enzyme inhibitors occurs only gradually in contrast to the acute inhibition of the plasma renin-angiotensin system. Ultimately, it may be the inhibition of the tissue angiotensin converting enzyme which determines the clinical efficacy of the treatment.

If a tissue renin-angiotensin system is present in the heart, then captopril may exert direct effects on myocardial performance. Possibly as a result of the masking effects on pre- and afterload, this had never been demonstrated in patients. In order to exclude these indirect effects, we studied the effects of captopril in the isolated rat heart during ischemia and reperfusion (Appendices 3, 4, 6, and 8). These studies corroborate that the heart has a local angiotensin converting enzyme which may be responsible for both the synthesis of locally generated angiotensin II and the catabolism of locally generated bradykinin. In the heart the renin-angiotensin system and the kallikrein-kinin system interact with each other and with other locally generated vasoactive substances by

autocrine and paracrine mechanisms. The ultimate outcome of these interactions plays an important role in several (patho)physiological conditions. Although the role of the cardiac renin-angiotensin system has received much attention during the recent years, almost nothing is known about the presence of a local kallikrein-bradykinin system and its potential role in the development of heart disease. Local stimulation of prostaglandin I2 and release of endothelium-derived relaxing factor may be enhanced by this system. There appears to be a balance between the effects of angiotensin II and bradykinin, which is influenced by captopril.

The interference by captopril with the angiotensin converting enzyme in the heart and vascular wall may have important consequences. In Appendix 3 we demonstrated concentration-dependent protection by captopril against reperfusion injury in the isolated rat heart. In this study captopril markedly reduced the incidence and duration of ventricular fibrillation upon reperfusion after 15 minutes of local ischemia produced by coronary artery ligation. As was shown by the reduced purine overflow, myocardial cells were protected against reperfusion damage. Noradrenaline overflow was reduced at all concentrations. Similar effects were present following administration ramipril (=HOE 498) but only when the active, angiotensin converting enzyme-inhibiting form was given. In contrast, all effects were abolished by simultaneous administration of indomethacin. This is shown in Appendix 4. Stimulation of prostacyclin synthesis by reduction of bradykinin breakdown, which is the result of angiotensin converting enzyme inhibition, apparently plays an important role in the protective effects of captopril. Although inhibition of locally generated angiotensin II may be an underlying mechanism, this could not be proven. In Appendix 6 part of the beneficial effects could be demonstrated when captopril was given just before reperfusion. This led to the speculation that scavenging of free radicals by its sulfhydryl group may contribute to the reduction in reperfusion damage. This was later corroborated by other investigators.

The next question was whether or not these effects were relevant in vivo. To answer this question we developed a closed-chest pig model. Ischemia and reperfusion were induced by inflation and deflation of a balloon at the end of an angioplasty catheter which was positioned via the left carotid artery in the anterior descending branch of the left coronary artery. Parallel to the isolated rat heart, effects on hemodynamics, on release of noradrenaline, and on tissue damage were measured following administration of captopril. Two weeks later we studied the inducibility of ventricular arrhythmias in the surviving pigs by programmed electrical stimulation. The results are shown in Appendices 5 and 6.

When captopril was administered continuously (Appendix 5), we again found a concentration-dependent protective effect on myocardial damage. There was a significant reduction during reperfusion in the release of creatine

kinase levels and in the purine overflow. Maximal noradrenaline overflow diminshed dose-dependently. In contrast to the experiments in the isolated rat heart, ventricular fibrillation upon reperfusion was not seen in any of the pigs. Instead, a delayed accelerated idioventricular rhythm occurred in almost all pigs without significant differences between treated and untreated hearts. We also demonstrated reduction of creatine kinase levels without any effect on this rhythm disturbance when captopril was administered in a single bolus injection prior to reperfusion (Appendix 6). However, after two weeks a reduction in inducibility of ventricular arrhythmias was demonstrated in the captopril-treated animals. This suggests that early intervention may have beneficial effects on the development of ventricular arrhythmias late after myocardial infarction. Moreover, a reduction in inducibility was demonstrated in untreated animals when captopril was given intravenously prior to programmed electrical stimulation.

These results indicated that the cardioprotective effects of captopril may have clinical relevance. To study this, we performed a clinical study with captopril in patients with acute myocardial infarction who were treated with thrombolytic therapy. The aim of this treatment is to dissolve the clot in an obstructed coronary artery leading to restoration of blood flow. Although it has been shown that early reperfusion may reduce myocardial infarct size with improvement of myocardial function and mortality, paradoxically reperfusion may sometimes cause additional myocardial damage. Therefore, there is a rationale for concomitant use of other agents to reduce this reperfusion damage and to augment the already beneficial effects of thrombolytic therapy. On the basis of our experimental results, captopril appeared to be such an agent.

The results are shown in Appendix 7. The aim of the study was primarily to establish a safe dose. Furthermore, we studied the effects on noradrenaline release and reperfusion arrhythmias. A severe decrease in blood pressure occurred when captopril was given intravenously, even at a very low concentration. Although no definite explanation was found, it was speculated that an interaction between captopril and streptokinase was involved. Following oral administration, the hypotensive effect was much less pronounced and comparable with changes in blood pressure in the control group. In accordance with our animal experiments, an accelerated idioventricular rhythm occurred frequently in all groups. Interestingly, we did find a reduction in the incidence of non-sustained ventricular tachycardia in the captopril treated patients compared to a historic control group. Noradrenaline levels decreased significantly after oral captopril. This may suggest that at least some of our experimental data can be extrapolated to the clinical situation but more studies are needed.

The final part of this thesis addresses the question whether captopril may have additional advantages compared to other angiotensin converting enzyme-inhibitors due to the presence of a sulfhydryl group in its molecule. The

possible role as a free radical scavenger has already been mentioned. The sulfhydryl moiety may also be responsible for differences in coronary vasodilating effects which we found in the isolated rat heart. Availability of sulhydryl groups is essential for the relaxation of vascular tissue by the endogenously present nitrovasodilator nitric oxide, which is identical to EDRF. This also applies to the vasodilating properties of exogenously administered nitrates which act via the formation of intermediates, among which nitric oxide. Investigation was carried out in the isolated rat heart, in which we compared the coronary vasodilating effects of captopril and ramiprilat, alone and in combination with isosorbide dinitrate (Appendix 8). Only captopril increased coronary flow and potentiated the vasodilating effects of isosorbide dinitrate. Similar effects were obtained when cysteine, another sulfhydryl containing compound with angiotensin converting enzyme-inhibiting properties, was given.

Thus, the presence of a sulfhydryl group may render captopril advantageous in patients with ischemic heart disease, especially in combination with nitrate therapy. This was investigated in our last study, Appendix 9. Ten patients with stable exercise-induced angina pectoris, while on maintenance treatment with a high dose of isosorbide dinitrate, received at random in a double-blind, cross-over design a single dose of captopril or placebo. A significant improvement in exercise testing following captopril treatment was found without significant differences in blood pressure and heart rate compared to the placebo. A sulfhydryl-dependent interaction with nitrate therapy might be responsible for this therapeutic effect and could have therapeutic implications.

In summary, this thesis shows that captopril and other angiotensin converting enzyme inhibitors are more than only inhibitors of the circulating renin-angiotensin system, prescribed for the treatment of hypertension and congestive heart failure as a result of their vasodilating properties. Due to the presence of components of both the renin-angiotensin system and the kinin-kallikrein system in the local tissues, pharmacological responses to angiotensin converting enzyme inhibitors are different to those originally thought. This especially applies to the heart, where local inhibition of the angiotensin converting enzyme resulted in cardioprotective effects during ischemia and reperfusion. Moreover, captopril has unique pharmacological properties due to its sulfhydryl moiety which may be responsible for therapeutic efficacy in angina pectoris, especially when combined with nitrate therapy.

A word of caution is warranted. In the animal experiments high concentrations of captopril were sometimes used, and our clinical studies do not allow definite conclusions with regard to clinical efficacy. Dose-finding and comparative studies, both with placebo and with other angiotensin converting enzyme inhibitors, are needed and will be performed. Notwithstanding, the first clinical studies are hopeful and, as also can be concluded from the literature,

there are many reasons to assume a beneficial role of captopril in the treatment of ischemic heart disease in general, and of myocardial infarction in particular. Ongoing and future trials will hopefully prove this assumption to be right!

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APPENDIX 1

ACUTE HEMODYNAMIC AND HORMONAL EFFECTS OF RAMIPRIL IN CHRONIC CONGESTIVE HEART FAILURE AND COMPARISON WITH CAPTOPRIL

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Summary

Acute hemodynamic and hormonal responses to ramipril in comparison with captopril were studied in 10 patients with moderate to severe congestive heart failure in an open, randomized study. Both drugs were given to 5 patients each in 2 increasing doses on 2 successive days. After 5 mg of ramipril angiotensin converting enzyme activity was significantly decreased during 24 hours with a maximum decrease 4 hours after administration. Mean arterial blood pressure decreased from 84 ± 5 to 62 ± 5 mmHg at 4 hours and 71 ± 4 mmHg at 12 hours, respectively, after this dose. Capillary wedge pressure decreased from 19 ± 1 mmHg to 13 ± 1 mmHg at 4 hours with a maximum increase in cardiac output from 3.8 ± 0.3 liters/min to 4.4 ± 0.3 liters/min at 2 hours. No significant cardiac effects were present 8 hours after administration. After 10 mg of ramipril, cardiac and hormonal effects showed a quicker onset of action and longer duration compared with the 5 mg dose. Mean arterial pressure decreased to 61 ± 6 mmHg. Similar effects were seen after captopril, but with a significantly shorter duration. Mean arterial pressure decreased from 82 ± 4 mm Hg to 64 ± 5 mmHg after 12.5 mg and to 58 ± 6 mmHg after 25 mg of captopril.

In patients with congestive heart failure ramipril has the hemodynamic profile of a long-acting and potent angiotensin converting enzyme inhibitor. Significant cardiac effects are present during 4 to 8 hours and angiotensin converting enzyme activity is still significantly inhibited 24 hours after a single dose of ramipril. Severe hypotension may occur in the acute phase; therefore, the starting dose should be ≤ 2.5 mg and further therapy should be individualized.

Introduction

Inhibition of the angiotensin converting enzyme may have a salutary effect on left ventricular performance in patients with congestive heart failure. [1] Captopril, the first orally active angiotensin converting enzyme inhibitor, has been shown in many trials to relieve symptoms of congestive heart failure and to improve cardiac hemodynamics, functional capacity and exercise tolerance. [2,3] Identical effects were seen with enalapril, a longer acting angiotensin converting enzyme inhibitor without the sulfhydryl group of captopril. [4-6] Although these drugs are generally well tolerated, severe symptomatic hypotension may occur after the first dose; [7,8] therefore, a low starting dose is recommended.

Ramipril is a new orally active angiotensin converting enzyme inhibitor. [9-11] From pharmacodynamic data in normal volunteers, it was predicted that a dose of only 5 mg or at most 10 mg would be adequate to treat patients with hypertension or congestive heart failure. [10] This was corroborated by preliminary experiences with ramipril in hypertensive patients. [11]

The aim of this study was to determine the acute hemodynamic and hormonal effects of ramipril in patients with congestive heart failure in comparison with captopril. The effects of both 5 and 10 mg of ramipril were studied to find an optimal starting dose, with particular reference to the hypotensive effect.

Methods

Ten patients with moderate to severe congestive heart failure (New York Heart Association functional class III to IV) participated in this open phase 2 study. The study protocol was approved by the ethical committee of Groningen University Hospital. We excluded patients with acute myocardial infarction or unstable angina pectoris within the preceding 4 to 6 weeks, systolic blood pressure < 90 mmHg and severe valvular disease or symptomatic ventricular arrhythmias. All patients gave their informed consent to the study. Ramipril was randomly assigned to 5 patients (ages 62 to 75 years) with an average ejection fraction, as determined by radionuclide ventriculography, of 20 $\pm$ 4%. The cause of the heart failure was ischemic heart disease in all patients. Five other patients (ages 47 to 81 years) with an ejection fraction of 27 $\pm$ 5% received captopril. Idiopathic congestive cardiomyopathy was found in 3 of these patients and ischemic heart disease in 2.

All patients were hospitalized in the general ward to obtain a stable condition for at least 3 days. Vasodilating drugs were discontinued while treatment with diet (3 to 5 g NaCl), diuretics and digoxin was maintained. After an overnight fast the patients were transferred to the coronary care unit. If possible, diuretics were stopped 24 hours in advance. A triple-lumen Swan-Ganz ther-

modilution catheter was placed in the pulmonary artery percutaneously via the subclavian vein. Right atrial, pulmonary artery and pulmonary capillary wedge pressures were recorded and cardiac output was measured by the thermodilution technique. Systemic arterial blood pressure was measured continuously by cannulation of the radial artery. Mean arterial pressure was estimated as diastolic pressure plus one-third of the difference between systolic and diastolic pressure. Systemic vascular resistance was calculated as follows: 80 [(mean arterial pressure - right atrial pressure)/cardiac output].

When a stable hemodynamic condition was achieved (variability between 3 control values of the cardiac output < 10 percent), the first oral dose of the angiotensin converting enzyme inhibitor was given; it consisted of 5 mg ramipril or 12.5 mg captopril. Hemodynamic parameters were measured hourly up to 8 hours after the first dose and at 4-hour intervals thereafter. After 24 hours a double dose, i.e., 10 mg ramipril or 25 mg captopril, was given and the same hemodynamic measurements were repeated.

To investigate the relation between the immediate hemodynamic effects and the effects of the drug on the renin-angiotensin-aldosterone system, blood samples were drawn from the arterial line before, and 1, 4, 8 and 24 hours after the first and second dose. Established techniques were used to determine plasma renin activity [12] and plasma levels of angiotensin converting enzyme, [13] aldosterone [14] and noradrenaline. [15]Routine blood chemistry was measured by autoanalyzer.

Statistical analysis was performed using analysis of variance. Differences were considered significant at a p value less than 0.05. Since age, ejection fraction and underlying disease were not equally distributed between the 2 groups, statistical analysis was performed only within the groups at different time points compared with baseline values. Results are given as mean values ± standard error of the mean.

Results

Hemodynamic effects (Figures 1 and 2)

Compared with 12.5 mg captopril, the onset of action after 5 mg ramipril was slower and the duration significantly longer. An initial decrease in blood pressure after the first dose of ramipril was noted after 1 hour, but peak changes occurred only after 3 to 6 hours and a significant effect was still present after 12 hours. This contrasted with a time to peak effect of 1 to 1.5 hours seen after 12.5 mg captopril and a total duration of action of 3 to 4 hours. When the dose of ramipril was doubled maximal effects were seen after 1 to 4 hours with a significant decrease in blood pressure up to 16 hours. After both captopril and ramipril individual responses varied; some patients showed a maximum res-

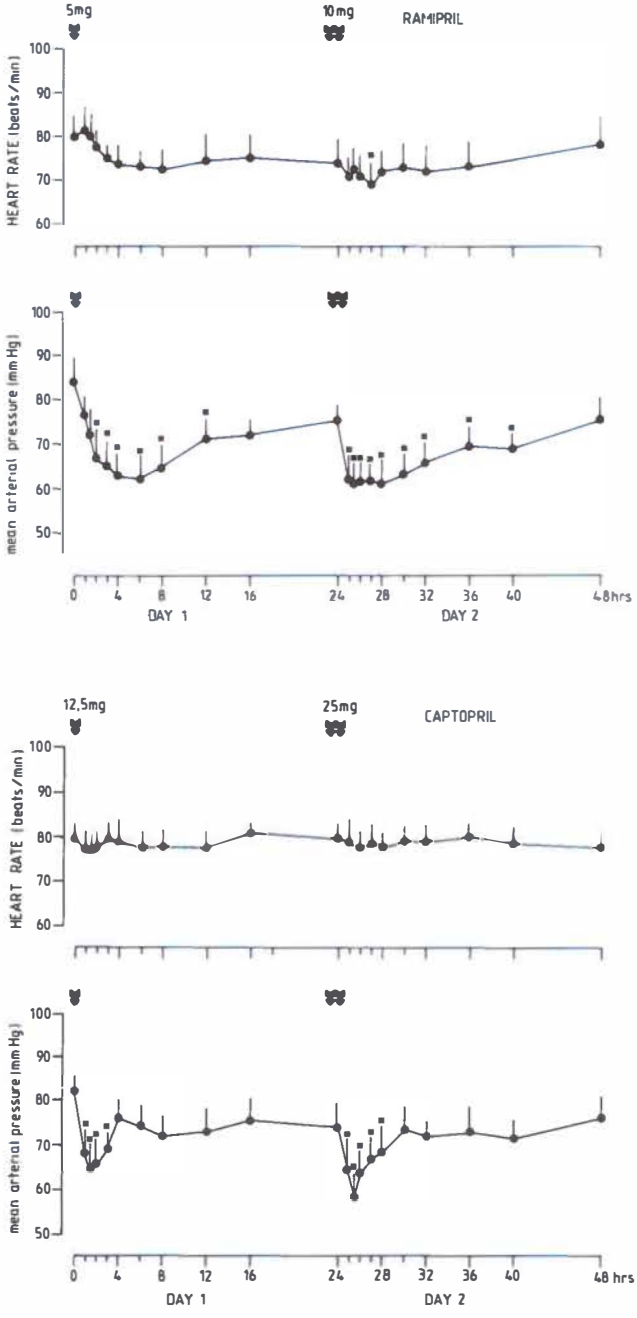

Figure 1. Changes in heart rate and mean arterial pressure after single doses of 5 and 10 mg ramipril, A, in comparison with 12.5 and 25 mg captopril, B. Values are mean ± SEM. *$p < 0.05$, compared with baseline values.

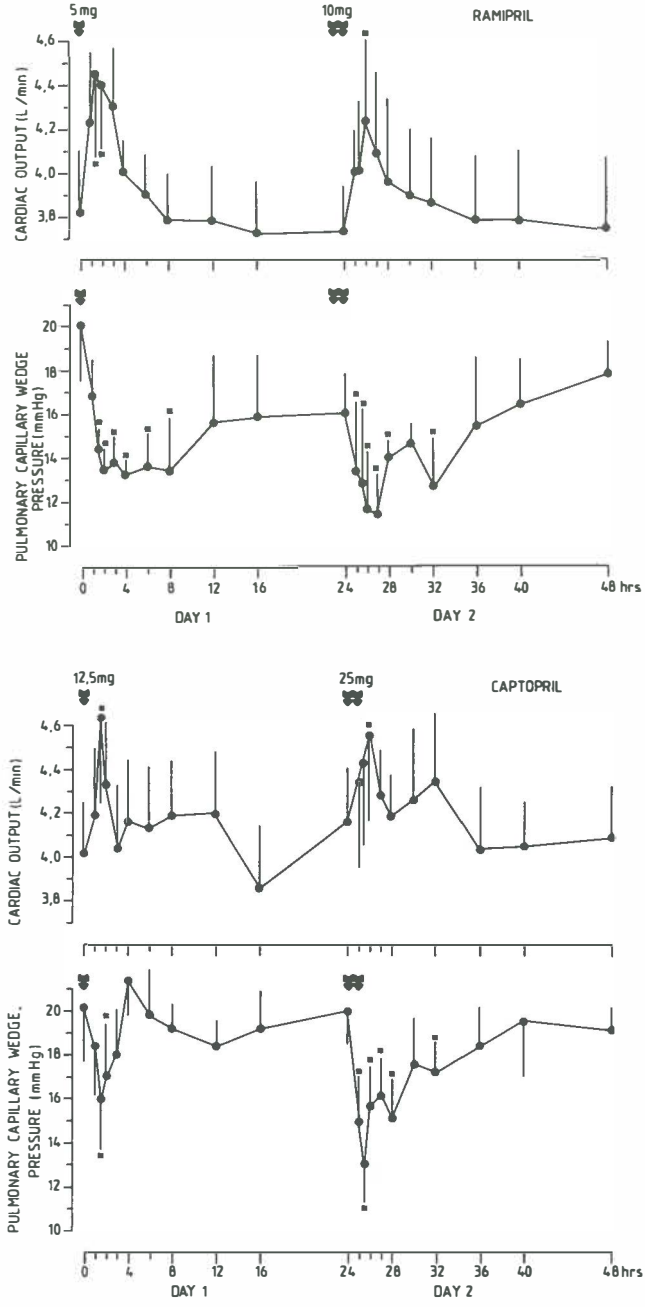

Figure 2. Changes in cardiac output and pulmonary capillary wedge pressure after single doses of 5 and 10 mg ramipril, A, in comparison with 12.5 and 25 mg captopril, B. Values are mean ± SEM. *$p < 0.05$, compared with baseline values.

ponse after the first dose and others after the second dose (Table 1). Despite this marked decrease in blood pressure no reflex tachycardia occurred and heart rate was even reduced in both groups at the time of maximum hemodynamic effects.

Table I. Individual data on pretreatment levels of serum sodium and plasma renin activity (PRA) and on maximal changes in mean arterial blood pressure

				Mean arterial pressure (mm Hg)		
Pt (n)	Treatment	Serum Sodium (nmol/liter)	PRA (nmol AI/1/hr)	Pretreatment	Day 1	Day 2
1	R	139	10.4	77	52	50
2	R	131	2.2	72	49	48
3	R	138	3.5	81	51	64
4	R	141	1.6	98	77	73
5	R	136	8.7	92	57	42
6	C	123	5.8	76	45	45
7	C	138	0.8	89	73	61
8	C	137	5.3	77	58	53
9	C	138	4.2	92	76	79
10	C	134	8.8	76	59	48

Patients 1 to 5 received 5 mg ramipril (R) on the first day and 10 mg R on the second day. Patients 6 to 10 received 12.5 mg captopril (C) on the first day and 25 mg C on the second day.

The percentage of decrease in systemic vascular resistance was similar to the decrease in mean arterial pressure. Maximum reduction was 31% and 32% after 5 and 10 mg ramipril, respectively, versus 26% and 33% after 12.5 and 25 mg captopril, respectively. Administration of both ramipril and captopril resulted in a significant increase in cardiac output, which was not dose-dependent. Although the effect of ramipril was prolonged compared with captopril, it lasted no longer than 8 hours after the first dose and 12 hours after the second. Pulmonary capillary wedge pressure showed a marked decrease in both groups with significant effects up to 8 hours after ramipril and values still below baseline after 24 hours. Similarly right atrial pressure dropped from 3.2 ± 0.9 mmHg to 1.6 ± 0.9 mmHg 4 hours after 5 mg ramipril and to 1.6 ± 0.7 mmHg 6 hours after 10 mg of ramipril. However, these changes were not significant. The same effect was seen after captopril, however again with a shorter duration.

Hormonal effects (Figure 3)

Plasma renin activity at baseline was elevated in most patients [Table 1, normal range 1.66 ± 0.99 (1 standard deviation)]. Concentrations increased signi-

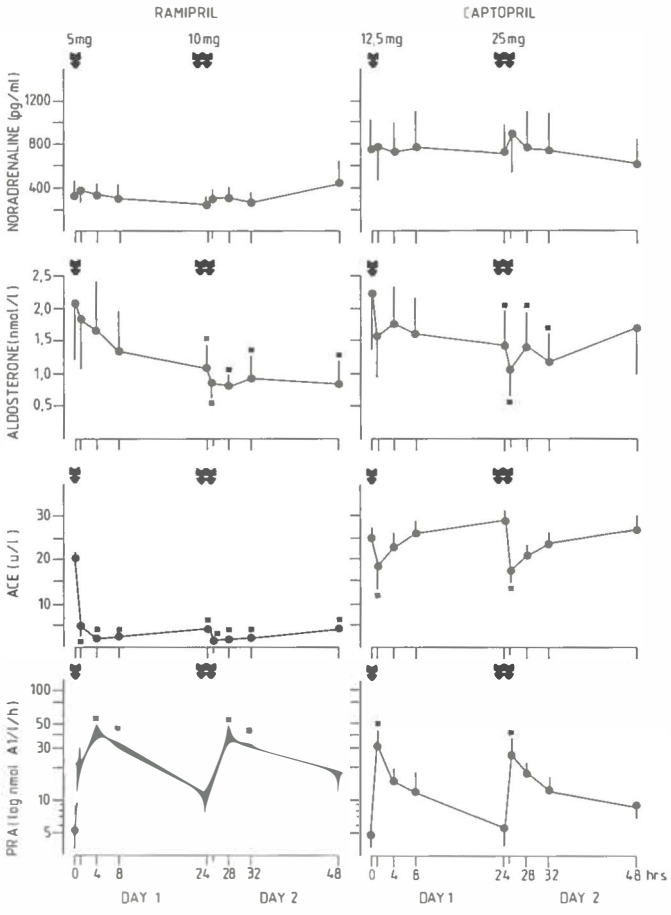

Figure 3. Changes in angiotensin converting enzyme (ACE), plasma renin activity (PRA), aldosterone and noradrenaline after 5 and 10 mg ramipril, left, in comparison with 12.5 and 25 mg captopril, right. Values are mean ± SEM. *$p < 0.05$, compared with baseline values.

ficantly after both drugs with comparable peak levels already after the first dose and significant longer duration after ramipril. No significant changes were present after 24 hours, although mean levels after administration of ramipril were still elevated compared with baseline values. In contrast, a significant inhibition of angiotensin converting enzyme activity was present throughout the 24 hours both after 5 and 10 mg ramipril with a decrease of more than 90% after 4 hours and approximately 80% after 24 hours. Inhibition of angiotensin converting enzyme activity was much less pronounced after captopril, but this may be due to a dissociation of the inhibitor enzyme complex in vitro, as has been described. [16] Both after captopril and ramipril a gradual decline of serum

aldosterone was seen with significant effect present during the second day. Despite the pronounced hemodynamic changes, no significant increase in noradrenaline was noted.

Adverse effects

Despite the marked and sometimes dramatic decrease in mean arterial pressure no significant side effects occurred. In 5 patients the hypotensive response was accompanied by complaints of dizziness or blurred vision, which lasted up to 5 minutes after captopril and up to 30 minutes after ramipril. Four of these patients had the highest levels of plasma renin activity (Table 1, patients 1, 5, 6 and 10). However, no significant correlation was found between the magnitude of blood pressure decrease after the first dose and the pretreatment level of plasma renin activity ($r = 0.34$), neither was there an inverse correlation between serum sodium and plasma renin activity before treatment ($r = -0.11$). No neurologic complications or angina pectoris were observed and renal function remained stable in all patients.

Discussion

This study demonstrates that ramipril in patients with moderate to severe congestive heart failure has the hemodynamic profile of a potent and long-acting inhibitor of angiotensin converting enzyme. In comparison with captopril a significantly longer duration of action was present with a slower onset and longer time to reach maximal effects.

This delayed peak response appears to correlate with the formation of the active metabolite, ramiprilat, after hydrolysis in the liver. [9,10] Correspondingly, a prolonged inhibition of angiotensin converting enzyme activity and an inversely related increase in plasma renin activity was noted. However, despite a reduction of angiotensin converting enzyme activity of approximately 80%, no significant cardiovascular effects were present after 24 hours. Apparently angiotensin converting enzyme inhibition of $\geq$ 90% is necessary to achieve significant effects on cardiac output and left ventricular filling pressure. This is in accordance with earlier findings on another long-acting angiotensin converting enzyme inhibitor, enalapril, and is probably related to the finding that at least 90% reduction of angiotensin converting enzyme-activity is necessary to induce effective blockade of the renin system. [9,10,17] However, it is still possible that once daily administration during long-term treatment is sufficient to exert hemodynamic effects during 24 hours, as has been demonstred with enalapril. [6]

The principal adverse effect of converting enzyme inhibition in patients with congestive heart failure is systemic hypotension. [18] Blood pressure declines in

nearly every patient who receives an angiotensin converting enzyme inhibitor for the first time, but these decreases are generally well tolerated. A consistent feature is the lack of tachycardia and, related to this, the failure of plasma noradrenaline to increase, as was also shown in our study. Despite sometimes marked hypotension, half of our patients were completely asymptomatic. When complaints of blurred vision and dizziness were present, these lasted longer after ramipril compared with captopril due to ramipil's longer duration of action, but no serious neurologic sequelae occurred. In contrast to other studies, [1,7] we did not find a significant correlation between pretreatment plasma renin activity and short-term hemodynamic response, although symptomatic hypotension did occur mainly in the patients with the most marked activation of the renin-angiotensin sytem. As with other angiotensin converting enzyme inhibitors, first dose hypotension after ramipril may unpredictably occur in patients with congestive heart failure, even when diuretics are withheld. Therefore, therapy must be started under close clinical observation and the inital dose should be lower than 5 mg, perhaps $\leq$ 2.5 mg. With reference to the variable dose-response effect, dose should only be increased slowly and carefully.

Both captopril and ramipril show beneficial effects on myocardial performance. Acute symptomatic relief was achieved in some of our patients, especially when signs of pulmonary congestion were present. Since these effects appear to decrease after 8 to 12 hours, a twice- daily dosage regimen may be necessary to obtain optimal therapeutic effect in these patients. There is now increasing evidence, however, that in other patients with only mild to moderate congestive heart failure acute hemodynamic determinations are of little value in predicting long-term clinical response. [19,20] This may be due to the fact that the improvement in functional capacity is more related to alterations in the distribution of peripheral blood flow, especially in skeletal muscle, than to changes in cardiac hemodynamics. [21,22] Because a substantial period is required before peripheral blood flow distribution may improve, it will take a few weeks before an optimal cinical response has been reached. In these patients a once-daily dosage regimen of ramipril may suffice. Treatment with ramipril in patients with congestive heart failure should therefore be individualized, depending on the clinical symptoms of the patients.

From our results we conclude that ramipril may be expected to be an effective drug in the treatment of congestive heart failure. Due to its hypotensive effect the starting dose should be as low as possible - $\leq$ 2.5 mg - and further treatment should be titrated depending on the clinical symptoms. Long-term, controlled clinical studies will have to determine whether its potent and long-acting angiotensin converting enzyme inhibition will prove of advantage in the expanding group of angiotensin converting enzyme inhibitors.

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Appendix 2

ACUTE AND CHRONIC EFFECTS OF RAMIPRIL AND CAPTOPRIL IN CONGESTIVE HEART FAILURE

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Summary

We studied the acute and long-term effects of ramipril and captopril in 12 patients with moderate to severe congestive heart failure using an open, parallel design. Drug doses were titrated. Compared with baseline values, maximal hemodynamic and humoral effects after the first dose of both angiotensin converting enzyme inhibitors were similar, but the effects of ramipril (5 mg) demonstrated a slower onset of action and a significantly longer duration than captopril (12.5 mg). After 3 months of treatment a single dose of 5 mg ramipril showed the same 24-hour hemodynamic profile as after the first dose, but the hypotensive effect was less marked. There was no plasma accumulation of ramiprilat. Serum creatinine and potassium remained stable, except for one patient whose renal function deteriorated on captopril treatment. Complex ventricular arrhythmias occurred in 11 patients and were unaffected after treatment with ramipril or captopril. Two patients died suddenly during ramipril therapy and one patient during captopril therapy.

In summary, ramipril is an effective, long-acting angiotensin converting enzyme inhibitor, producing long-term hemodynamic effects in patients with congestive heart failure. Using an individualized dosing scheme, neither long-lasting hypotension nor deterioration of renal function occurred. No effect on ventricular arrhythmias was seen.

Introduction

Oral angiotensin converting enzyme inhibitors occupy an increasingly important place in the treatment of congestive heart failure. Consistent circula-

tory and symptomatic improvement has been shown, superior to the responses seen during therapy with placebo or other vasodilator drugs. [1] Furthermore, experimental and clinical data suggest that converting enzyme inhibitors exert favorable effects on the survival in these patients. [1-4] A reduction of life-threatening ventricular arrhythmias may contribute to this effect. [3]

Ramipril is a new orally active angiotensin converting enzyme inhibitor with a long duration of action. [5] Only few studies have been done in patients with congestive heart failure [6-9] and little is known about the hemodynamic effects during long-term use, or concurrent metabolic, hormonal and renal changes. It has been suggested that long-acting angiotensin converting enzyme inhibitors may produce prolonged hypotensive effects which may compromise cerebral and renal function, and thus have disadvantages, as compared with short-acting agents such as captopril. [10]

We studied the hemodynamic and hormonal responses to both acute and chronic therapy with ramipril. Preliminary data on the acute effects in comparison with captopril have already been published. [7] Changes in blood pressure and renal function were compared to those after captopril during long-term treatment. In addition, the effects of both drugs on the severity of ventricular arrhythmias were monitored by 24-hour ambulatory monitoring.

Methods

Patient selection

Thirteen patients (1 female and 12 male) with chronic congestive heart failure were selected in this open parallel study. All patients had severe restriction of physical activity or symptoms of dyspnoe or fatigue at rest for more than three months (New York Heart Association functional class III-IV), despite adequate treatment with salt restriction, diuretics and digoxin. Their ages ranged from 48 to 81 years with a mean of 64 ± 3 years. All patients gave their informed consent before entering the study. Patients with acute myocardial infarction or unstable angina pectoris within the preceding six weeks, systolic bloodpressure ≤ 90 mm Hg, severe valvular disease and a creatinin clearance less than 30 ml/min were excluded. Ramipril was randomly assigned to seven patients and captopril to six patients. The cause of heart failure was ischemic heart disease in all ramipril-treated patients and in three captopril-treated patients. Idiopathic congestive cardiomyopathy was diagnosed in the other three captopril-treated patients. One patient turned out to have hyperthyroidism after six weeks of captopril treatment and was excluded from the evaluation of the results.

Study design

All patients were hospitalised. Vasodilator therapy was discontinued at least 24 hours before the start of the study. Treatment with salt restriction (3-5 g NaCl), diuretics and digoxin was maintained. Subsequently basic clinical non-invasive parameters were determined. Following this, right heart catheterisation was performed with a Swan-Ganz triple-lumen thermodilution catheter. The radial artery was cannulated for continuous blood pressure monitoring. When a reproducible cardiac output was achieved (averaging at least three replicate determinations varying less than 10%), 5 mg ramipril or 12.5 mg captopril was given orally with hemodynamic monitoring for 24 hours. After completion of these measurements, doses were doubled and a maintenance dose of 10 mg ramipril once daily or 25 mg captopril three times daily was given, unless symptomatic hypotension occurred. After reaching a clinically stable condition, patients were discharged and seen as out-patients on a regular basis every three weeks. When clinical response was unsatisfactory, the dose was adjusted to a maximum of 10 mg ramipril twice daily or 50 mg captopril three times daily. Total study duration was 12 weeks. At the end of the study patients who had been treated with ramipril underwent further invasive hemodynamic measurements during 24 hours after a single dose of 5 mg ramipril, using the same protocol as after the first dose. Because sustained hemodynamic effects of captopril had already been demonstrated by several other investigators, [1, 10, 11] it was considered unnecessary and unethical to repeat this in the captopril-treated patients.

Parameters

Symptoms were evaluated by assessing the New York Heart Association score. Ambulatory electrocardiographic monitoring was performed during 24 hours, before treatment and after six and 12 weeks. To classify ventricular arrhythmias, the modified Lown grading system was used: grade 0, no ventricular premature beats; grade 1, isolated ventricular premature beats ($\leq$ 30 per hour); grade 2, frequent ventricular premature beats ($\geq$ 30 per hour); grade 3, multiform ventricular premature beats; grade 4A, repetitive ventricular premature beats (couplets); grade 4B, repetitive ventricular premature beats (salvos or nonsustained ventricular tachycardia). [12] Invasive hemodynamic recordings were resting heart rate, mean arterial pressure, cardiac output by thermal dilution (in triplicate), and pulmonary capillary wedge pressure. Blood pressure was also measured non-invasively at each visit with a conventional mercury sphygmomanometer. Mean arterial blood pressure was defined as diastolic pressure plus one-third of the difference between systolic and diastolic pressure. Left ventricular ejection fraction was recorded by gated radionuclide technique before the study and after three months of treatment.

Venous blood was drawn before and at 1, 3, 6, 9, and 12 weeks of therapy. Samples were assessed for serum electrolytes, urea and creatinine concentrations. During the initial hospitalisation 24-hour urine was collected daily for measurement of the creatinine clearance. Arterial blood samples were drawn before, and 1, 4, 8, and 24 hours after the dose, during the invasive hemodynamic measurements. Established techniques were used to determine plasma renin activity and plasma levels of angiotensin converting enzyme activity and aldosterone. [13-15] Concentrations of ramipril and its active metabolite ramiprilat were determined by means of radioimmunoassay before and 0.5, 1, 2, 3, 4, 8, and 24 hours after the dose [16] [detection limits 0.5 and 0.2 ng/ml, respectively].

Statistical Analysis

Statistical evaluations were done using the Student t test for paired observations (hemodynamics) and the Wilcoxon test for paired data (biochemical and neurohumoral changes). Differences were considered significant at $p \leq 0.05$, two-sided. Since age, initial hemodynamic data and underlying disease were not equally distributed between the two groups, statistical analysis was performed only within the groups at different time points compared with the baseline values. Results are given as mean values ± standard error of the mean.

Results

Clinical data of the patients before entry are listed in Table 1. Average ejection fraction was 26 ± 4% in the ramipril-treated group and 27 ± 4% in the captopril-treated group. The dose of captopril was increased in three patients to 25 mg three times daily and in one patient to 50 mg three times daily, whereas one patient tolerated only 12.5 mg twice daily. Ramipril was incremented in 3 patients to 10 mg once daily and in one patient to 10 mg twice daily; two patients received only 5 mg once daily as a maintenance dose because of symptomatic hypotension. Eight patients completed the study. Ramipril was discontinued in one patient after the first dose of 5 mg because of a catheter mediated infection. Two patients died suddenly following 1 and 10 weeks of treatment with ramipril, after having shown initial clinical improvement. One patient, who had been treated with captopril, was withdrawn from the study after 9 weeks of treatment because of progression of the heart failure. This patient died suddenly one week later.

Signs and Symptoms

At the end of the study or before discontinuation 2 patients in the captopril group and 4 in the ramipril group showed symptomatic improvement of one

Table 1

Clinical data of the patients studied with captopril (C) or ramipril (R). Symptoms were classified according to the New York Heart Association (NYHA), before treatment and at the last visit. Ejection fraction (EF) was measured before and after 3 month's therapy.

Pt	Rx	age	sex	diagnosis	NYHA-class		EF	
No.		(years)			before	after	before	after
1	R	68	M	IHD	III-IV	III	21%	26%
2	R	62	M	IHD	III	II	23%	23%
3	R	75	M	IHD	III-IV	II	41%	41%
4	R	62	M	IHD	IV	III	10%	12%
5	R	70	F	IHD	IV	III	31%	--[b]
6	R	75	M	IHD	III	III	16%	--[b]
7	R	76	M	IHD	III-IV	--[a]	39%	--[b]
8	C	48	M	PCM	III	II	22%	59%
9	C	81	M	IHD	III	III	45%	40%
10	C	50	M	IHD	III	II	22%	22%
11	C	58	M	PCM	III-IV	IV	28%	42%
12	C	52	M	IHD	III-IV	IV	17%	--[b]

IHD = ischemic heart disease; PCM = primary (congestive) cardiomyopathy. M = male; F = female.

[a] D/C after first dose due to catheter sepsis.

[b] No EF after 3 months due to premature discontinuation of the study.

class of the New York Heart Association criteria (Table 1). Changes in ejection fraction did not parallel this symptomatic improvement, with significant increase (≥ 10%) in only 2 patients (Table 1).

Hemodynamics

Acute effects (Figure 1, left panel). Compared to baseline values maximal hemodynamic effects of both angiotensin converting enzyme inhibitors were similar. A marked drop in blood pressure was seen with an increase of cardiac index, a decrease of pulmonary capillary wedge pressure and a reduction in heart rate. However, the effect of ramipril on hemodynamic parameters showed a slower onset of action with a significantly longer duration than captopril. After ramipril reduction of blood pressure occurred and was maximal after 3-4 hours and lasted up to 24 hours, whereas after captopril this was 1.5-2 hours and four hours respectively. After both drugs the blood pressure lowering effect persisted much longer than the effects on cardiac index and capillary wedge pressure.

Chronic effects (Figure 1, right panel). Invasive hemodynamic effects were only measured in the ramipril-treated group of four patients who finished the

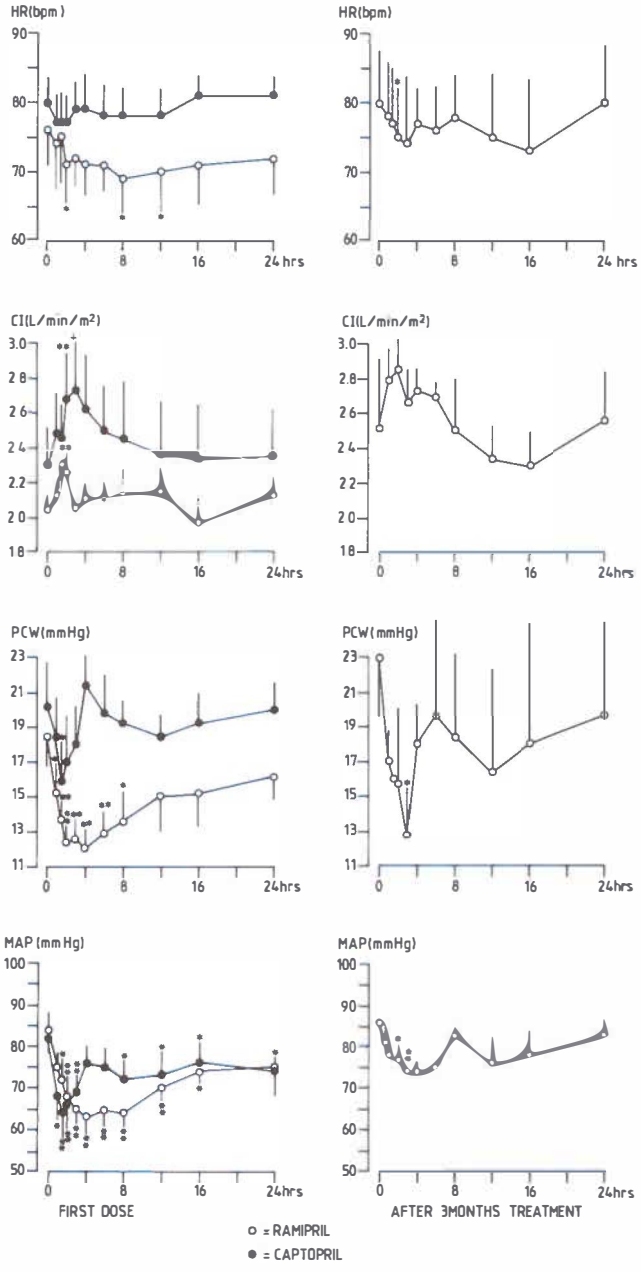

Figure 1. Changes in heart rate (HR), mean arterial pressure (MAP), capillary wedge pressure (PCW) and cardiac index (CI) after the first dose of 5 mg ramipril and 12.5 mg captopril (left panel) and after 5 mg ramipril following three months of treatment (right panel). Symbols are mean ± SEM.
*$p \leq 0.05$; **$p \leq 0.01$, compared with values at t= 0

study. In one of these patients cardiac index and capillary wedge pressure could not be measured due to refusal of the patient to undergo right heart catheterisation. Hemodynamic changes after three months of treatment with ramipril showed the same initial values and the same 24-hour profile as before and after the first dose although the hypotensive effect was less pronounced. The time of maximal effect was also the same. When measured non-invasively, mean arterial blood pressure at the last visit was 92 ± 2.5 mm Hg in the ramipril-treated group and 88 ± 2.5 in the captopril-treated group, not significantly different from baseline values.

Ambulatory monitoring

According to the modified Lown classification complex ventricular arrhythmias (Lown grades 3, 4A and 4B) were present in 11 of 12 patients before treatment (Table 2). Four patients received antiarrhythmic therapy, which was continued during the study. After treatment no significant changes occur-

Table 2.

Ventricular arrhythmias according to the modified Lown grading system in patients with congestive heart failure (CHF) treated with captopril (C) and ramipril (R), recorded by 24-hour ambulatory electrocardiography before treatment and after 6 or 12 weeks (last recording).

Pt	Rx	Rhythm	Lown grading system		Remarks
nr			before	after	
1	R	SR	III	IVA	
2	R	SR	IVB	IVA	disappearance non-sustained VT
3	R	AF	IVB	IVA	disappearance non-sustained VT
4	R	SR	III	IVA	
5	R	SR	IVB	IVB	† after 10 weeks "sudden death"
6	R	PM	IVA	---	† after 1 week "sudden death"
7	R	AF	IVB	---	after 1 day D/C due to sepsis
8	C	AF	I	IVB	
9	C	AF	III	III	
10	C	SR	IVB	IVB	VT 1.6 sec → 2.2 sec frequency 150 → 160 bpm
11	C	PM	IVB	IVB	VT 1.6 sec → 3.2 sec frequency 200 → 120 bpm
12	C	PM	IVB	IVB	VT 1.2 sec → 1.8 sec after 10 weeks D/C due to CHF

SR = sinusrhythm; AF = atrial fibrillation; PM = pacemaker; VT = ventricular tacycardia; VPB = ventricular premature beat;
I = isolated VPB's; III = multiform VPB's; IVA = repetitive VPB's (couplets); IVB = repetitive VPB's (salvo's or nonsustained VT); bpm = beats per minute.

red. Two patients changed from grade 4B to 4A (disappearance of non-sustained ventricular tachycardia), two from grade 3 to 4A and 1 deteriorated from grade 1 to grade 4B (Table 2).

Biochemical and hormonal changes

At baseline serum potassium was within the normal range in all patients. During the study no hypokalaemia (serum K $\leq$ 3.5 mmol/l) or severe hyperkalaemia (serum K $\geq$ 6.0 mmol/l) occurred. At the end of the study mean values were not significantly changed compared to the initial values (Table 3). Creatinine clearance at the start of the study varied from 27-107 ml/min in the ramipril group and 36-96 ml/min in the captopril group. Mean values of serum creatinine and urea did not change significantly in both treated groups and the individual data demonstrate a mild improvement in most patients (Table 3). In the

Table 3.

Serum creatinine, urea and potassium before and after treatment with captopril or ramipril at the last visit.

Pt nr	creatinine (μ mol/l)		urea (mmol/l)		potassium (mmol/l)	
	before	after	before	after	before	after
Ramipril						
1	141	124	12.5	7.1	4.0	3.9
2	110	99	11.9	6.3	3.9	3.9
3	109	130	9.1	8.7	4.2	4.2
4	132	122	15.8	8.4	4.2	4.5
5	107	96	10.9	20.4	3.7	4.4
6	94	91	8.6	7.4	4.1	4.2
7	142	–	9.4	–	3.7	–
x	119	115	11.2	9.7	3.97	4.1
	±7	±6	±0.9	±2.0	±0.07	±0.11
Captopril						
8	110	85	5.2	4.1	4.6	5.2
9	159	157	12.9	11.0	4.0	4.1
10	84	94	7.5	6.5	4.3	4.8
11	131	361	12.3	35.7	3.8	3.9
12	139	101	22.5	8.0	5.6	5.4
x	125	160	12.1	13.1	4.46	4.5
	±11	±46	±2.7	±5.2	±0.28	±0.21

D/C after first dose due to catheter sepsis.

ramipril-treated group one patient showed a marked increase in serum urea only (case 5) and in the captopril-treated group one patient demonstrated an increase in both serum urea and creatinine (case 11).

Neurohumoral changes are shown in Figure 2. After the first dose there was significant inhibition of angiotensin converting enzyme activity and increase of

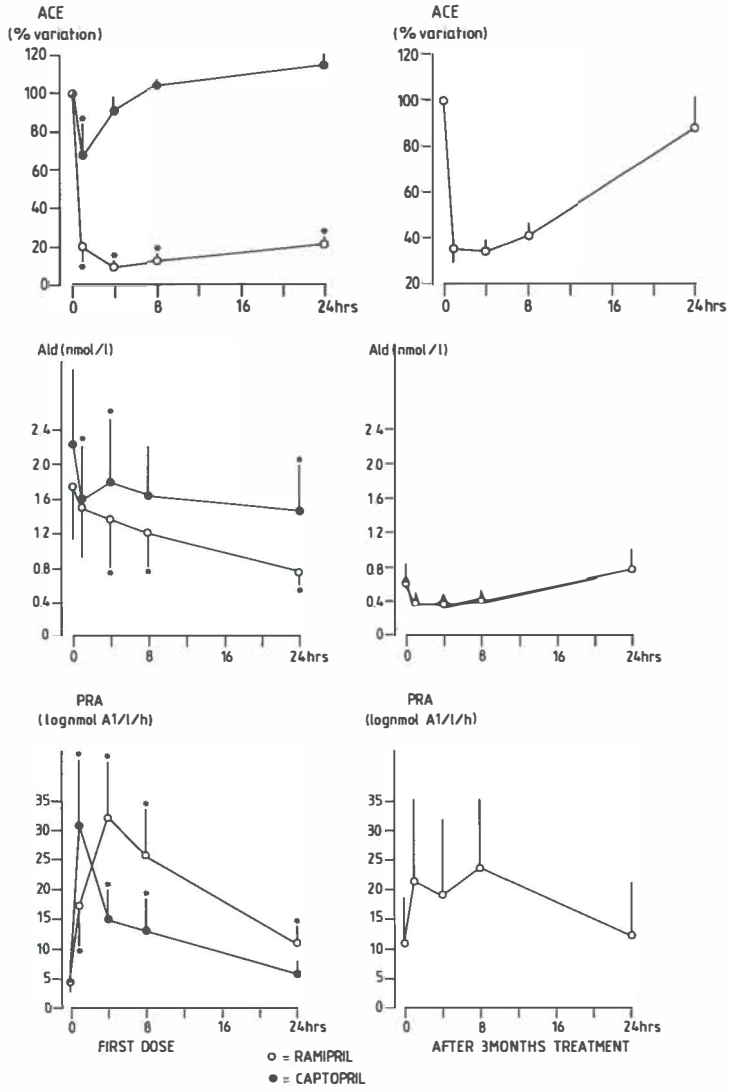

Figure 2. Changes in angiotensin converting enzyme (ACE), plasma renin activity (PRA) and aldosterone (Ald) after the first dose of 5 mg ramipril and 12.5 mg captopril (left panel) and after 5 mg ramipril following three months' treatment (right panel). Symbols are mean ± SEM.
*$p \leq 0.05$; **$p \leq 0.01$, compared with values at $t = 0$

plasma renin activity up to 24 hours after ramipril and up to eight hours after captopril. Inhibition of angiotensin converting enzyme activity was much less after captopril, which may, at least partly, be due to a dissociation of the inhibitor enzyme complex in vitro. Aldosterone levels showed a more gradual decline. After 12 weeks these changes were already present before the morning dose of ramipril in comparison to the situation before treatment, but a 24-hour profile with maximal effects after 1-4 hours was maintained. Angiotensin converting enzyme levels were 20 ± 1 U/l before the first dose and 7 ± 1 U/l before the dose after three months.

Plasma levels

Plasma levels of ramipril and ramiprilat were measured in four patients after a single dose of 5 mg ramipril following three months of treatment (Figure 3). No accumulation had occurred since basic levels were either very low or non-detectable. Levels of ramipril peaked after 0.5-2 hours and fell rapidly. The levels of the active diacid (ramiprilat) showed maximal values after 1-3 hours with a more gradual decline, in concurrence with the hemodynamic changes.

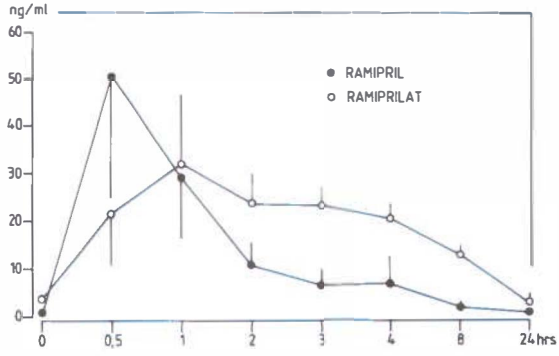

Figure 3. Plasma levels of ramipril and ramiprilat after a single dose of 5 mg ramipril following three month's treatment. Symbols are mean ±SEM.

Adverse events

During the acute phase experiments three patients developed symptomatic hypotension with dizzines, blurred vision and sleepiness. This lasted approximately 15 minutes during ramipril (n = 2) and 5 minutes during captopril (n = 1) with a good response to intravenous saline. During the chronic study both captopril and ramipril were tolerated well. Only one patient developed itching and a mild rash during captopril treatment, which spontaneously subsided.

Discussion

This study shows that ramipril produces both short-term and long- term hemodynamic and symptomatic improvement. As has been demonstrated with other angiotensin converting enzyme inhibitors, no tolerance develops for these effects. [11,17] Ramipril clearly has the profile of a long-acting angiotensin converting enzyme inhibitor and significant inhibition of the angiotensin converting enzyme is present over at least 24 hours, comparable with enalapril. [18] Values of the angiotensin converting enzyme after three months treatment before the daily dose still show a 65% inhibition, similar to those seen 24 hours after the first dose. Nevertheless, hemodynamics were unchanged compared to values before the first dose and significant changes only occurred after the ramipril was given, with the same 24-hour profile as during the acute phase. It has been shown that a ramipril diacid metabolite plasma concentration of approximately 5 ng/ml is required to achieve 80% inhibition of plasma angiotensin converting enzyme and thereby to induce significant effects on peripheral flow. [19] In our study lower levels were detected before and 24 hours after the dose. No accumulation in multiple dose conditions seems to occur, as is also demonstrated in healthy volunteers. [20] This leads to submaximal angiotensin converting enzyme suppression before the next dose and, subsequently, to a characteristic 24-hour hemodynamic profile after the following dose.

This raises the question whether the aim of the treatment with ramipril should be a more persisting effect during 24 hours. If so, the dose should be higher or should be given at least twice daily. It has been shown with enalapril that when large doses are given, divided over the day, symptomatic hypotension and subsequent decline in creatinine clearance are more likely to occur. [10] This led to the statement that continous blockade of angiotensin I formation can be detrimental. In our study, where the dose was adjusted to the signs and symptoms of the patient, both captopril and ramipril were tolerated well, despite the fact that marked hypotension, sometimes symptomatic, did occur after the first dose. Serum creatinine deteriorated only in one patient during captopril therapy and remained stable in the ramipril-treated group. Therefore, it may be advantageous to maintain the 24-hour profile and to include a period of submaximal angiotensin converting enzyme inhibition. However, the starting dose should have been smaller, for instance 1.25 mg for ramipril and 6.25 mg for captopril, which was well tolerated generally in another study in patients with congestive heart failure. [8]

Although our study shows that ramipril can have comparable beneficial effects to captopril, it should be mentioned that an unequal distribution of age, initial hemodynamic data and underlying disease was present between the two groups. All ramipril-treated patients had ischemic heart disease as cause for

their heart failure, while this was the case in only three out of five captopril-treated patients. Interestingly, a marked increase in ejection fraction was seen only in the two captopril-treated patients with primary cardiomyopathy, but this coincided with an improvement of the clinical situation in only one patient and only a small number of patients was studied. It remains to be established, whether differences in the cause of heart failure will influence the response to different angiotensin converting enzyme inhibitors.

Despite hemodynamic improvement following treatment with ramipril, two patients died suddenly after one and 10 weeks, respectively. In the captopril-treated group one patient was removed from the study after ten weeks due to progression of the heart failure. This patient died suddenly one week later. The occurrence of sudden death did not appear to be treatment related. Patients with moderate to severe congestive heart failure have a very poor prognosis and sudden death occurs in many. [3,21,22] Related to this high mortality is the development of complex ventricular arrhythmias, when left ventricular function deteriorates. [3,21,22] It has been shown by several investigators that angiotensin converting enzyme inhibition might beneficially influence the incidence and duration of these arrhythmias [23-25] and it was suggested that this may improve survival. [3,22] However, we could not detect significant changes in ventricular arrhythmias with captopril or with ramipril. The reason remains unclear,but it may be due to the fact that serum potassium was in the normal range in all patients both before and during the study, whereas in the other studies hypokalaemia ($K \leq 3.5$ mmol/l) was present in a considerable number of patients before treatment was started and serum potassium increased significantly during treatment. Hypokalaemia has been associated with ventricular arrhythmias, especially when digoxine is used. [21] In a recent placebo-controlled study with enalapril, a significant reduction in mortality at the end of one year was noted in the actively treated group, but no significant reduction of sudden death. [4] No information is available about serum potassium levels, although hypokalaemia occurred more often in the placebo group as compared to the enalapril group. Therefore, it remains to be proven whether angiotensin converting enzyme inhibitors have anti-arrhythmic properties when serum potassium is normal and whether this will beneficially influence the incidence of sudden death.

In summary, ramipril is an effective, long-acting angiotensin converting enzyme inhibitor, producing long-term hemodynamic effects in patients with congestive heart failure. The first dose should be given once daily and the ultimate dose titrated to the patient's needs. Further studies are needed to ascertain the effect on ventricular arrhythmias.

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APPENDIX 3

CONCENTRATION-DEPENDENT PROTECTION BY CAPTOPRIL AGAINST ISCHEMIA-REPERFUSION INJURY IN THE ISOLATED RAT HEART

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Summary

The purpose of this study was to investigate the protective effects of captopril at three concentrations (1, 10 and 80 μg/ml) against reperfusion injury after 15 minutes of coronary ligation in the isolated perfused rat heart. During ischemia, the apex displacement and pressure rate index decreased markedly and only in the presence of 80 μg/ml captopril less reduction of the pressure-rate index was obtained ($p < 0.05$). Upon reperfusion, the apex displacement and pressure-rate index improved significantly in all captopril-treated hearts, depending on the concentration used, whereas the untreated hearts showed a further deterioration ($p < 0.05$). Ventricular fibrillation upon reperfusion occurred in 6/6 untreated hearts, but only in 4/6 hearts at 1 μmg/ml, in 2/6 hearts at 10 μg/ml and 0/6 hearts at 80 μg/ml captopril with a significantly shorter duration ($p < 0.05$). In control hearts a marked overflow of purines and norepinephrine was found in the coronary effluent upon reperfusion. In contrast, captopril decreased purine overflow, most at 80 μg/ml, and norepinephrine levels were undetectable at all concentrations.

These results indicate concentration-dependent protective effects of captopril after local ischemia and reperfusion, already present at therapeutic levels. Reduction of cellular injury and suppression of norepinephrine release appear to play an important role in the improvement of mechanical function and the reduction in reperfusion arrhythmias.

Introduction

In recent years it has been demonstrated that captopril treatment yields beneficial effects in patients with severe congestive heart failure. [1-3] Captopril, a

competitive inhibitor of the angiotensin converting enzyme, improves cardiac performance as a result of a reduction in systemic vascular resistance and the various determinants of left ventricular filling pressure. [3]

Apart from these mechanisms it is unclear whether captopril has a direct effect on myocardial performance, independent of pre- and afterload reduction. We described additional beneficial effects of captopril in the isolated rat heart after reversible ligation of the left coronary artery. [4] Captopril reduced ventricular fibrillation and the loss of high energy phosphate nucleotides and thereby partly maintained mechanical function impaired by ischemia and reperfusion. However, we used captopril in a high concentration (80 μg/ml).

Therefore, the present study was designed to investigate whether these beneficial effects are also present at levels which are therapeutic in man. Moreover, we measured norepinephrine overflow in the coronary effluent in order to gain an insight into the underlying mechanisms.

Methods

Preparation of the heart

Male Wistar rats (275-325 g), fed ad libitum, were anesthetized with ether and given 500 I.U. of heparin intravenously. Hearts were rapidly removed and cooled in ice-cold 0.9% NaCl. Retrograde perfusion of the aorta as described by Langendorff at a constant pressure of 60 mm Hg was started immediately using a modified Tyrode solution. [4] The hearts beat spontaneously. Temperature was kept between 36.5 and 37.5 °C.

Acute regional myocardial ischemia was produced by occlusion of the left coronary artery. This was accomplished by tightening a previously placed ligature round the descending branch 2 mm below the aortic root. The myocardium was reperfused by releasing this ligature.

Protocol

After 15 minutes of equilibration the experiments were started with a control period of 15 minutes. Subsequently, acute myocardial ischemia was evoked during 15 minutes. Then the coronary ligature was released and measurements were obtained for 30 minutes after reperfusion.

The rat hearts were randomised into four groups of 6 each. Captopril was given during the whole experiment to three groups at a concentration of 1, 10, or 80 μg/ml. One group served as control. Fresh solutions were prepared daily.

Coronary flow

Coronary flow was measured by a microprocessor, which maintained the perfusion pressure at 60 mm Hg by adjusting the peristaltic perfusion pump (LKB, microperpex).

Electrophysiological parameters

A bipolar cardiac electrogram was obtained by a silver electrode, which was attached to the ventricular apex and the metal inflow cannula was used as the indifferent electrode. Heart rate and the occurrence of arrhythmias were derived from this electrogram.

Mechanical parameters

The apex displacement, left ventricular pressure with its derivative dP/dt and the pressure-rate index were used as parameters of contractility. Left ventricular pressure was measured by means of a catheter in the left ventricle, which was connected to a pressure transducer (Statham P23 Db). The dP/dt, which is defined as the ratio of the change in pressure to the change in time, was registered at the same time as the left ventricular pressure, both during contraction and relaxation. The pressure-rate index was calculated as the product of left ventricular pressure and heart rate. Apex displacement was measured with an isotonic displacement transducer (Hottinger Baldwin, W10) fitted with a steel tampon hooked to the apex of the ventricle. All parameters were calculated as a percentage of the values at the end of the equilibration period ($t = 0$).

Biochemical assays

The total overflow of the purine adenosine and its catabolites (inosine, hypoxanthine and xanthine) was measured in the coronary effluent and used as a sensitive indicator for nucleotide breakdown. [5] One minute samples were collected at $t = 0$ and at 15, 30, 31, 35, 45 and 60 minutes. The purines were assayed by high pressure liquid chromatography. [5] At the end of the experiments, the hearts were dried to constant weight and the purine overflow was expressed as nmol/minute/gram dry weight (gdwt). The samples were also assayed for norepinephrine levels in order to quantify the overflow of this catecholamine. A sensitive HPLC method with electrochemical detection was used. [6] Simultaneously, determinations of angiotensin II were made in the coronary effluent of control hearts by radioimmunoassay with a detection limit in plasma of 2 pg/ml. [7]

Statistical analysis

Statistical analysis was performed with the Student t test or the Mann Whitney U test if the assumption of a normal distribution had to be rejected. Differences were considered significant at a p value of less than 0.05, two-sided. Results are given as mean values $\pm$ SEM.

Results

Coronary flow (Table 1)

At the end of the control period no significant differences in flow were present between the captopril-treated groups and the control group. Occlusion of the coronary artery resulted in a comparable flow reduction in all groups, sustained during the whole ischemic period. Upon reperfusion, an immediate and total recovery was present: 9.9 ± 0.3 ml/minute for control hearts and 10.3 ± 0.4 ml/minute, 10.6 ± 0.3 ml/minute and 10.4 ± 0.5 ml/minute for hearts treated with 1, 10 and 80 μg/ml, respectively. At the end of the reperfusion period coronary flow had somewhat decreased in all groups without significant differences.

Table I

Effects of 1, 10, and 80 μg/ml captopril on coronary blood flow and heart rate in the isolated rat heart during ischemia and reperfusion

		Captopril		
	Control	*1* μg/ml	10 μg/ml	80 μg/ml
	End Control Period (t = 15)			
Coronary flow (ml/min)	10.1 ± 0.4	9.8 ± 0.7	10.4 ± 0.3	10.7 ± 0.7
Heart rate (beats/min)	303 ± 9	300 ± 7	303 ± 8	287 ± 16
	End Ischemia (t = 30)			
Coronary flow (ml/min)	6.1 ± 0.5	6.9 ± 0.4	7.9 ± 0.4	7.6 ± 0.4
Heart rate (beats/min)	293 ± 15	288 ± 15	297 ± 9	272 ± 12
	End Reperfusion (t = 60)			
Coronary flow (ml/min)	9.4 ± 0.5	9.0 ± 0.6	10.2 ± 0.4	10.3 ± 0.5
Heart rate (beats/min)	287 ± 13	298 ± 14	295 ± 13	283 ± 20

Values are means ± SEM

Heart rate (Table 1)

No significant differences in heart rate were noted between the groups at the end of the control period. A slight, but insignificant decrease was noted during ischemia in all groups. After reperfusion, again no significant differences were present between the hearts when sinus rhythm was present.

Contractility (Table 2)

At the end of the control period none of the measured parameters of contractility was significantly changed by captopril. During ischemia contractility

Table II

Effects of 1, 10, and 80 μg/ml captopril on mechanical parameters in the isolated rat heart during ischemia and reperfusion

	Control	*Captopril* 1 μg/ml	10 μg/ml	80 μg/ml
End Control Period (t = 15)				
Apex displacement (mm)	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.2	1.1 ± 0.1
Left ventricular pressure (mmHg)	40 ± 4	39 ± 7	38 ± 5	44 ± 4
dP/dt contraction (mmHg/sec)	318 ± 30	300 ± 43	302 ± 38	330 ± 26
dP/dt relaxation (mmHg/sec)	210 ± 32	200 ± 34	196 ± 34	211 ± 29
End Ischemia (t = 30)				
Apex displacement (mm)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Left ventricular pressure (mmHg)	16 ± 2	21 ± 4	18 ± 4	31 ± 4*
dP/dt contraction (mmHg/sec)	126 ± 7	168 ± 24	120 ± 21	238 ± 28*
dP/dt relaxation (mmHg)sec)	77 ± 6	107 ± 17	78 ± 14	158 ± 21*
End Reperfusion (t = 60)				
Apex displacement (mm)	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1*
Left ventricular pressure (mmHg)	14 ± 8	37 ± 4 *	39 ± 6 *	51 ± 2 **
dP/dt contraction (mmHg/sec)	103 ± 65	277 ± 30*	308 ± 42*	392 ± 16*
dP/dt relaxation (mmHg/sec)	71 ± 45	202 ± 28*	218 ± 40*	272 ± 6*

Values are means ± SEM
*p<0.05; **p<0.01, as compared with control group

decreased markedly in all groups. Only administration of 80 μg/ml captopril resulted in significantly less reduction of the left ventricular pressure and dP/dt, but changes in apex displacement were the same as in other groups. After reperfusion, a further gradual decrease in mechanical parameters was seen in the control groups, whereas all captopril-treated hearts improved significantly to the end of this period. This is also demonstrated by the pressure-rate index, which shows a concentration-dependent beneficial effect of captopril on myocardial function (Figure 1).

Occurrence of reperfusion arrhythmias

During ischemia infrequent ventricular premature beats occurred in all hearts but no ventricular fibrillation was seen. Upon reperfusion all untreated hearts showed ventricular fibrillation. A marked reduction in these ventricular arrhythmias was seen when captopril was present in the perfusate. At a concentration of 1 μg/ml ventricular fibrillation occurred in four of six hearts, at 10 μg/ml in two of six hearts and at 80 μg/ml in none of the hearts. When ventricular fibrillation occurred in the captopril-treated hearts, a significantly shorter duration was noted (Figure 2).

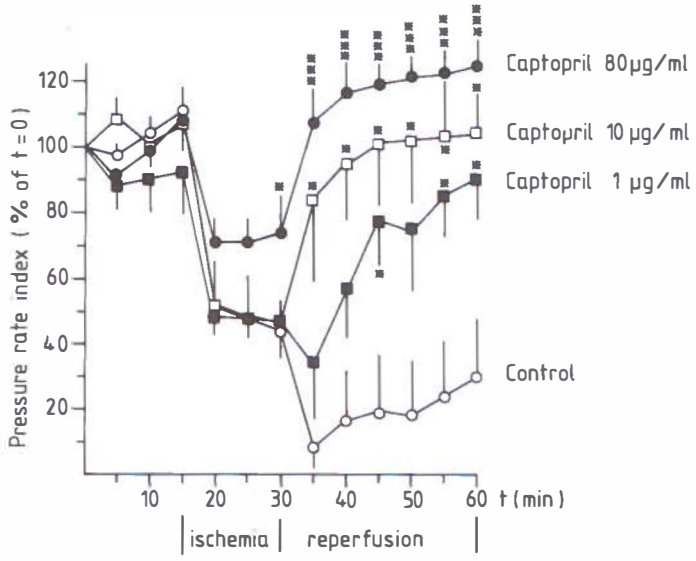

Figure 1. Time course of the pressure-rate index during ischemia and reperfusion in control hearts and captopril-treated hearts (1, 10 and 80 μg ml). Results are calculated as percentage of the values at t = 0. Values are mean ± SEM.
*p<0.05; **p<0.001, compared with the control group.

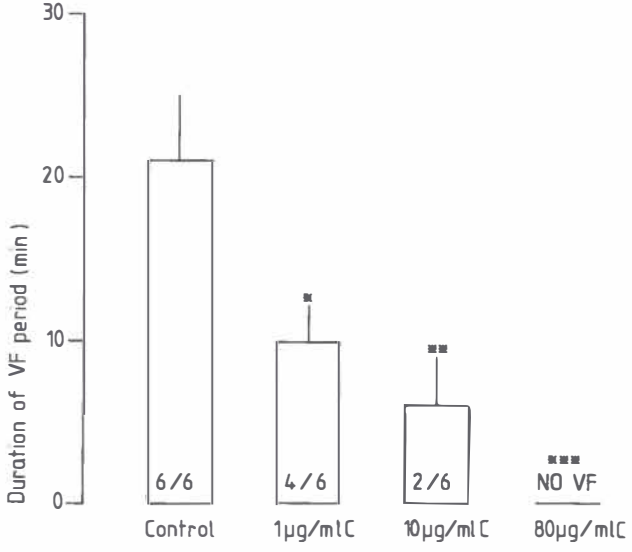

Figure 2. Duration and incidence of ventricular fibrillation upon reperfusion in control hearts and captopril-treated hearts (C) (1, 10 and 80 μg/ml). The open columns represent the mean duration of ventricular fibrillation (± SEM) and the numbers indicate the fraction of the hearts in which this occurred.
*p<0.05; **p<0.02; ***p<0.001, compared with the control group.

Cellular injury

The purine overflow in the coronary effluent is shown in Figure 3. During the control period no significant alterations by captopril were found. After co-

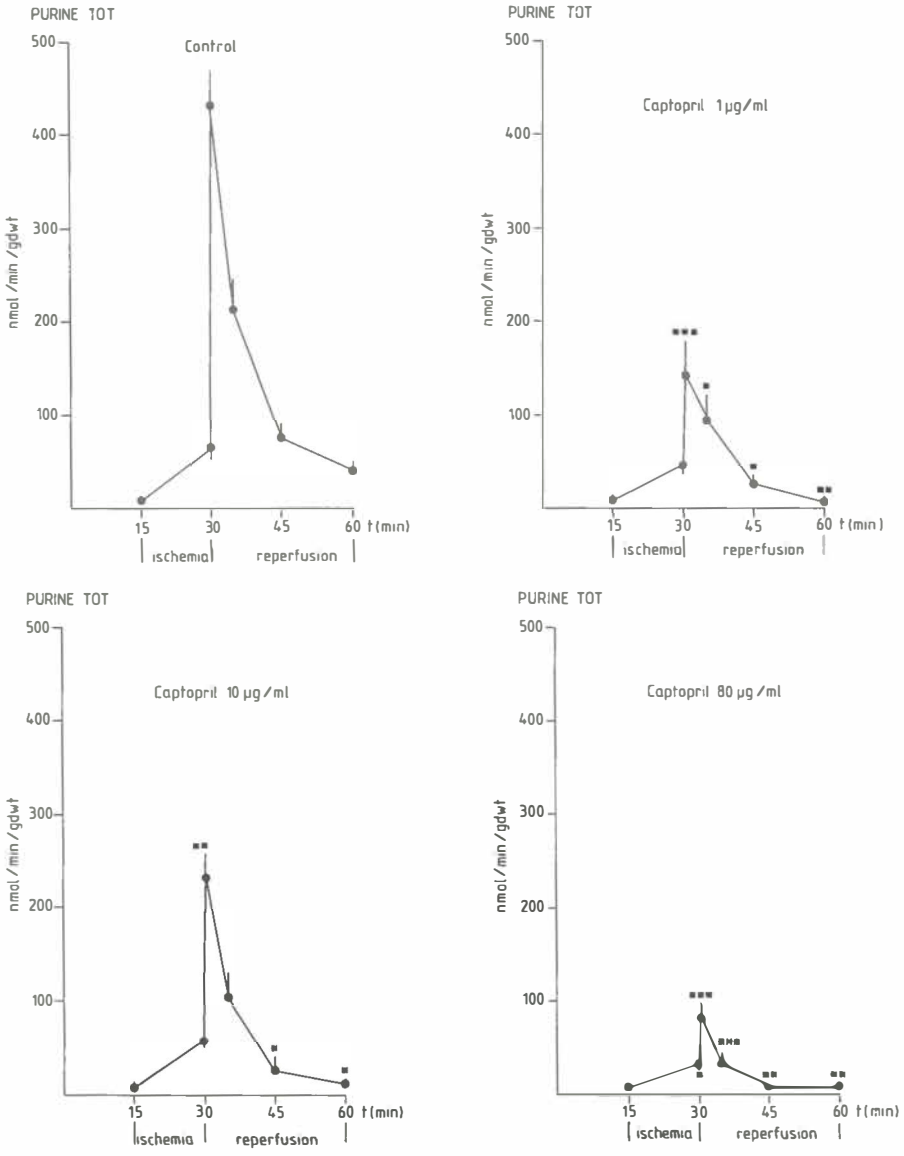

Figure 3. Time course of the purine overflow in the coronary effluent during ischemia and reperfusion in control hearts and captopril-treated hearts (1, 10 and 80 μg/ml). The measured values are calculated as nmol/minute and expressed per gram dry weight (gdwt). Values are mean ± SEM. *p<0.05; **p<0.01; ***p<0.001, compared with the control group.

ronary ligation the concentration of purines increased in all hearts. Only in the 80 μg/ml captopril group a significant reduction was seen in comparison with the control group ($p < 0.05$). Upon reperfusion the largest increase in purine overflow was seen in the untreated hearts (431 ± 38 nmol/minute gram dry weight). This was significantly less in the hearts treated with 1, 10 and 80 μg/ml captopril (141 ± 9, 235 ± 23 and 82 ± 15 nmol/minute gram dry weight, respectively). Throughout the reperfusion period purine values were lowest in the 80 μg/ml group, followed by the 1 μg/ml group and finally the 10 μg/ml group.

Norepinephrine levels

No detectable norepinephrine was present in the coronary effluent at the end of the control period and at the end of the ischemic period (detection limit 5 pg/ml). Following reperfusion, a marked rise in norepinephrine concentration was determined during the first minute in the untreated hearts. Subsequently, a rapid decline occurred and no measurable levels of norepinephrine were detected at the end of the reperfusion period (Figure 4a). However, when captopril was present in the perfusate, no overflow of norepinephrine was measured in any of the treated groups upon reperfusion (Figure 4b).

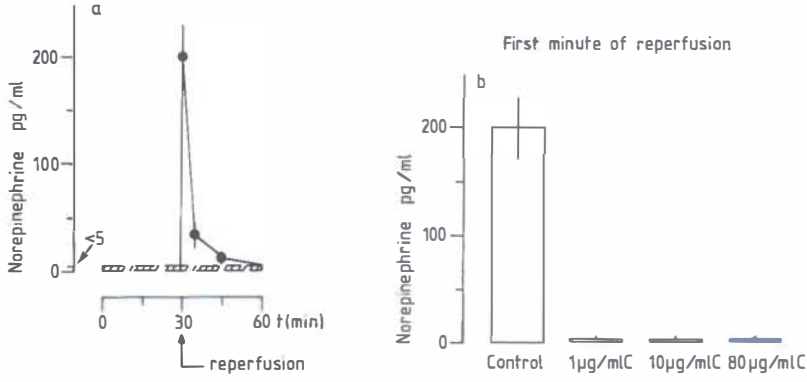

Figure 4.
a. Time course of the norepinephrine concentration in the coronary effluent during ischemia and reperfusion in untreated hearts (detection limit 5 pg/ml).
b. Concentration of norepinephrine in the coronary effluent during the first minute of reperfusion in control hearts and captopril-treated hearts (C) (1,10 and 80 μg/ml). Values are mean ± SEM.

Angiotensin II

No angiotensin II was detectable in the coronary effluent of control hearts during the whole experiment (detection limit 2 pg/ml).

Discussion

The present study shows that captopril protects the mechanical function of the heart during ischemia and reperfusion in a concentration-dependent way. Following ligation of the coronary artery, impairment of mechanical function was reduced only at high concentrations of captopril, as demonstrated by a significant increase of the pressure-rate index. However, after reperfusion the pressure rate-index and other parameters of contractility increased significantly already at low concentrations and further recovery of the contractility occurred when higher concentrations of captopril were used.

Concomitant with these findings a reduction of purine overflow was detected during reperfusion in all captopril-treated hearts. Purines are ATP catabolites and can be used as sensitive indicators for the nucleotide breakdown, which occurs after cellular ischemia. [5] Recovery of the cardiac mechanical function during reperfusion correlates with the tissue level of ATP. [8] Captopril apparently prevents the depletion of cardiac pools of nucleotides taking place during ischemia. This effect was most pronounced at the highest concentration of captopril, but lower levels were not linearly related to the amount of nucleotide breakdown. Apparently, the relation between nucleotide breakdown and impairment of mechanical function is complex; chronology of the various events and a differential effect of captopril concentrations on both phenomena may be involved.

The isolated rat heart subjected to coronary artery occlusion and reperfusion, has proven to be a useful model to provoke ventricular arrhythmias. [9] In our study all control hearts developed ventricular fibrillation upon reperfusion. This arrhythmia was prevented when captopril was added to the perfusate. Even at low concentrations a significant reduction of the incidence and duration of ventricular fibrillation was seen. This protective effect increased even further when higher doses of captopril were used. This confirms our preliminary observation of a beneficial effect of a high concentration of captopril (80 μg/ml) on reperfusion arrhythmias [4] and it demonstrates that this effect is concentration dependent and already present at levels which are within the therapeutic range. [10]

In order to elucidate the underlying mechanisms we measured norepinephrine levels in the perfusate, since it is known that ventricular arrhythmias correlate with increased activity of the sympathetic system. [11,12] We detected significant amounts of norepinephrine the first minute of reperfusion in all control hearts, but in none of the captopril-treated hearts. It appears that captopril reduces norepinephrine overflow upon reperfusion.

It has been demonstrated that captopril interferes with adrenergic transmission both via pre- and postjunctional actions. [13-18] At lower concentrations this effect appears to be mediated by a selective inhibition of angiotensin II in the

vasculature. [13,14,17] Angiotensin II can both increase norepinephrine release and facilitate the postjunctional effects of norepinephrine. [19,20] However, at higher doses an angiotensin-independent action of captopril has been reported leading to an attenuating effect on the vascular response to norepinephrine. [14,15,17,18] In our model it is unlikely that decrease of angiotensin II formation plays a role in the inhibitory effect on norepinephrine release. Although angiotensin II can be generated from angiotensin I by local angiotensin converting enzyme in the isolated heart, [21] this does not seem to occur in our experiments, since no angiotensin I appears to be present in the isolated perfused rat heart. This was corroborated by our experiments in untreated hearts, in which no angiotensin II was detected in the coronary effluent during ischemia and reperfusion.

Therefore, it appears that in our study captopril suppresses norepinephrine release in an angiotensin-independent way. A tentative mechanism for the reduction of both norepinephrine and purine overflow may be that captopril inhibits the leak of these intracellular chemicals through the cell membrane during ischemia and reperfusion by decreasing its permeability. However, this awaits further investigation.

It is still open to discussion whether this inhibitory effect on norepinephrine overflow plays a central role in the protective effect of captopril on reperfusion arrhythmias. It cannot be ruled out that a concentration-dependent relation with these arrhythmias is still present at very low norepinephrine levels. However, other mechanisms such as the improved metabolic status on reperfusion, the increase of myocardial high energy phosphate content and the reduction in lactate concentration have also been associated with protection against reperfusion ventricular fibrillation. [22] The role of adrenergic, biochemical and metabolic influences on reperfusion arrhythmias has not yet been clearly defined.

We conclude from these data that captopril protects against myocardial injury and ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated rat heart. Reduction of cellular damage and inhibition of norepinephrine overflow appear to be important underlying mechanisms. These concentration dependent effects are also present at therapeutic plasma levels and further in vivo studies are warranted in order to assess the clinical relevance of our findings.

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APPENDIX 4

REDUCTION OF REPERFUSION ARRHYTHMIAS IN THE ISCHEMIC ISOLATED RAT HEART BY ANGIOTENSIN CONVERTING ENZYME INHIBITORS. A COMPARISON OF CAPTOPRIL, ENALAPRIL, AND HOE 498.

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Summary

The effects of the angiotensin converting enzyme inhibitors, captopril, enalapril, HOE 498, and its prodrug on reperfusion arrhythmias after 15 minutes of coronary ligation were investigated in the isolated rat heart. Drug concentrations were equipotent in their effect on angiotensin I pressor response. Furthermore, the effect of indomethacin on angiotensin converting enzyme inhibition with captopril was studied. Upon reperfusion, ventricular fibrillation occurred in all untreated hearts, in all prodrug HOE 498-treated (15 μg/ml) hearts and in 4 of 6 of the enalapril-treated (8 μg/ml) hearts. In contrast, in only 2 of 6 ($p < 0.002$) of the HOE 498-treated (15 μg/ml) hearts and in none ($p < 0.001$) of the captopril-treated (80 μg/ml) hearts did ventricular fibrillation occur. A massive purine overflow was observed in untreated hearts upon reperfusion. This overflow was significantly reduced by captopril and HOE 498, whereas enalapril and prodrug HOE 498 had no significant effect. Concomitantly, the pressure-rate index was severely impaired after 30 minutes of reperfusion in the untreated, enalapril, and prodrug HOE 498 groups (33 ± 9, 52 ± 11, and 48 ± 12% of initial values, respectively), but captopril and HOE 498 significantly reduced the impairment of mechanical function (124 ± 9% and 98 ± 9%, respectively). In contrast to enalapril and prodrug HOE 498, captopril and HOE 498 markedly reduced noradrenaline overflow during the first minute of reperfusion. No angiotensin II was detectable in the coronary effluent of untreated hearts. All the beneficial effects of captopril were abolished by simultaneous administration of indomethacin (1 μM), and no significant differences were observed for these hearts when compared to untreated hearts.

It is concluded that captopril and HOE 498 protect against reperfusion arrhythmias. Apparently this effect is independent of inhibition of the formation of angiotensin II and is associated with an abolishment of noradrenaline overflow upon reperfusion. Stimulation of prostacyclin synthesis appears to play an important role.

Introduction

Elucidation of reperfusion phenomena has taken on new significance because of recent clinical evidence that sudden restoration of coronary blood flow can result in serious structural and functional derangements, leading to ventricular fibrillation. [1-4]

The role of adrenergic, biochemical, and metabolic influences on reperfusion-induced ventricular arrhythmias has not been clearly defined. However, it is evident that reperfusion arrhythmias are quite distinct from those associated with ischemia. [5] There appears to be a relationship between the onset of irreversible injury during the ischemic period and the occurrence of ventricular reperfusion arrhythmias. [6]

Recently, .we have reported beneficial effects of captopril in an isolated heart model of malignant reperfusion arrhythmias [7] It was proposed that this protective effect was caused by an angiotensin II independent mechanism. The demonstration that captopril may interfere directly with the neurogenic noradrenaline transmission [8] supports this hypothesis.

The present study was undertaken to investigate whether or not this protective effect of captopril is also present with other non-sulfhydryl angiotensin converting enzyme inhibitors, such as enalapril and HOE 498. These compounds are prodrugs which must be de-esterified first before they become pharmacologically active. In this study, the active diacid forms of enalapril and HOE 498 were used. In order to assess the role of angiotensin converting enzyme inhibiting activity, the prodrug of HOE 498 was also studied and angiotensin II levels were measured. Furthermore, noradrenaline release and its modulation by angiotensin converting enzyme inhibitors were studied. Recently, a direct stimulatory action of captopril on prostacyclin synthesis has been shown. [9] In order to investigate whether or not this mechanism plays a role in the earlier observed protective effects of captopril, [7] cyclooxygenase inhibition was used to test this hypothesis.

Methods

Reperfusion model

Male Wistar rats (275-325 g), fed ad libitum, were anesthetized with ether

and given 500 IU of heparin intravenously. The hearts were rapidly excised and arrested in ice-cold 0.9% NaCl. Retrograde perfusion of the aorta, as described by Langendorff, was immediately started with a modified Tyrode solution, [10] containing 10 mM glucose and equilibrated with 95% O_2 and 5% CO_2. This perfusion buffer was filtered through 1.2- μm pore size filters before reaching the heart. The perfusion pressure was maintained at 60 mmHg. The temperature was continuously measured in the aorta-cannula tip and was kept between 36.5 and 37.5 °C. The hearts beat spontaneously.

Acute regional myocardial ischemia was produced as described by Kannengieser et al. [11] The left main coronary artery was ligated with 6-0 silk 2 mm below the aortic root using a 3/8 circle taper point needle (Ethicon). Reperfusion of the ischemic tissue was achieved by releasing the ligation. This technique of reversible ligation of the left coronary artery has proven to be a useful model to provoke ventricular arrhythmias. [12,13]

Protocol

The hearts were allowed to equilibrate with the perfusion fluid for 15 minutes. After this equilibration period and a control perfusion of 15 minutes, local ischemia was induced and maintained for the next 15 minutes. After reperfusion of the ischemic zone for 30 minutes, the experiments were terminated.

The rat hearts were divided at random into groups of six each. Drugs were added at the start of the control period, and treatment was continued during the whole experiment, including the period of reperfusion. Drug concentrations in study A were: Captopril 80 μg/ml, enalapril 8 μg/ml, HOE 498 15 μg/ml, and prodrug HOE 498 15 μg/ml. Drug concentrations in study B were: captopril 80 μg/ml, indomethacin 1 μM, and one group received both captopril and indomethacin in the above mentioned concentrations. Both in study A and B, one group served as control.

Measurement of mechanical and electrophysiological parameters

The pressure-rate product was used as an index of contractility [14,15] and, hence, of oxygen consumption. [16,17] Left ventricular end-systolic pressure was measured by means of a catheter inserted into the left ventricle via the mitral valve and connected to a pressure transducer (Statham P23 Db). The pressure-rate index was calculated as the product of maximal left ventricular end-systolic pressure and heart rate. Pressure-rate data during the experiment were calculated as a percentage of the values at the end of the equilibration period. A bipolar cardiac electrogram was obtained by means of two electrodes: One attached to the metal inflow cannula and the other to the ventricular apex outside the ischemic zone. Heart rate, PQ interval, and the occurrence of arrhythmias were monitored by continuous registration of the cardiac electrogram in

orde to evaluate the electrophysiologic function of the heart. Electrocardiogram was visualized using an ink jet recorder at a paper speed of 100 mm/s (Siemens Oscillomink E). Coronary flow (volume of perfusion fluid per time unit) was measured by a microprocessor, which controlled the perfusion pressure by adjusting the peristaltic perfusion pump (LKB, microperpex).

Assay of adenosine triphosphatase (ATP) catabolites, catecholamines and angiotensin II

Overflow of adenosine and its catabolites (inosine, hypoxanthine and xanthine) was used as an indicator of nucleotide breakdown. This has proven to be a reliable parameter for the degree of ischemia-induced cellular damage. [13, 18] One-minute fractions of the perfusate were collected in ice-cold tubes during the whole experiment. The purine nucleosides and oxypurines were determined by a slightly modified version of the high-performance liquid chromatography assay, described by Harmsen et al. [19] Analysis of catecholamines was based on liquid chromatography and electrochemical detection, as described by Westerink. [20] Angiotensin II levels were measured in control hearts with a commercially available radioimmunoassay kit (IRE, Fleurus, Belgium). At the end of the experiment, the hearts were dried to constant weight, and the purine overflow was expressed as nanomols per minute per gram dry weight (g dwt).

Reagents

All chemicals were analytical grade. Captopril was a gift from Squibb; HOE 498 and its prodrug were a gift from Hoechst, and enalapril was a gift from MSD. Fresh solutions were prepared daily.

Statistical analysis

The data are expressed as mean values ± SEM. Group differences were tested with the Student t test or Mann-Whitney U test; p values less than 0.05, double-sided, were considered to be significant.

Results

Control perfusion period

At the end of the control period, none of the measured parameters was significantly altered by any treatment when compared to the control group. In this period, no noradrenaline and angiotensin II were detectable in the coronary effluent of both untreated and treated groups. Apparently, all hearts were in a similar condition at the start of the ischemic period.

Ischemic period

Ligation of the left coronary artery resulted in comparable flow reduction for the entire group of hearts to 59 ± 4% of control values. After 15 minutes of regional ischemia, the pressure-rate index of all groups was impaired. However, the pressure-rate index of the captopril-treated hearts was significantly less impaired than the pressure-rate index of the control hearts (Table I). All groups showed a slight, but insignificant, decrease in heart rate without a change of the PQ interval during coronary ligation. Purine overflow increased during coronary ligation in all groups. However, this increase of purine overflow was significantly less for the captopril group as compared to the untreated group. During the ischemic period no overflow of noradrenaline or angiotensin II was detectable in the coronary effluent in any of the investigated groups. All groups showed a slight increase in ventricular premature depolarizations after 10 minutes of ischemia. However, the incidence of these rhythm disturbances was low and not significantly different between the groups.

Table I. *Study A: Effects of captopril, enalapril HOE 498, and its prodrug on the pressure-rate index (% of t=0) at the end of the ischemic period (I) and at the end of the reperfusion period (R)*

	I	R
Untreated	44 ± 8	33 ± 9
Captopril	74 ± 11[a]	124 ± 9[a]
Enalapril	30 ± 14	52 ± 11
HOE 498	49 ± 9	98 ± 9[a]
Prodrug HOE 498	45 ± 5	48 ± 12
Study B: Effect of captopril, indomethacin, and captopril plus indomethacin on the pressure-rate index at the end of I and R.		
Untreated	44 ± 6	37 ± 9
Captopril	69 ± 3[a]	107 ± 8[a]
Indomethacin	39 ± 12	42 ± 6
Captopril plus indomethacin	37 ± 8	52 ± 10

[a] A significant change ($p < 0.05$) as compared to the untreated group.

Reperfusion period

Release of the coronary ligation resulted in an immediate and total recovery of the coronary flow in all groups. At the onset of reperfusion in study A, all untreated and prodrug HOE 498-treated hearts showed ventricular fibrillation for 21 ±4 and 17 ± 2 minutes, respectively. Four of the six enalapril-treated

hearts fibrillated upon reperfusion for 23 ± 4 minutes, which is not significantly different from the untreated group. In contrast, captopril and HOE 498 treatment significantly reduced the incidence and duration of ventricular fibrillation after restoration of flow. Ventricular fibrillation occurred in none of the captopril-treated hearts and in only two HOE 498-treated hearts for a period of 8 ± 2 minutes ($p < 0.001$ and < 0.002 compared to controls, respectively) (Figure 1).

At the onset of reperfusion in study B, results were obtained for the untreated and captopril-treated group that were similar to those of study A. The incidence of ventricular fibrillation in the untreated group was again 100%, with a duration of 7.8 ± 2.2 minutes, whereas captopril prevented ventricular fibrillation completely. The indomethacin-treated hearts invariably showed ventricular fibrillation upon reperfusion, for 4.2 ± 2.2 minutes, which is not significantly different from control values. Addition of indomethacin to the captopril-treated hearts caused the effects of captopril on reperfusion arrhythmias to disappear. All these hearts showed ventricular fibrillation upon reperfusion for a period of 3.3 ± 1.1 minutes, which is not significantly different from the untreated group in study B.

The effects on the pressure-rate index also showed marked group differences after 30 minutes of reperfusion. In the untreated, enalapril, and prodrug HOE 498 groups, no significant recovery of the pressure-rate index was observed when compared to the preceding ischemic period (Table I). In contrast, af-

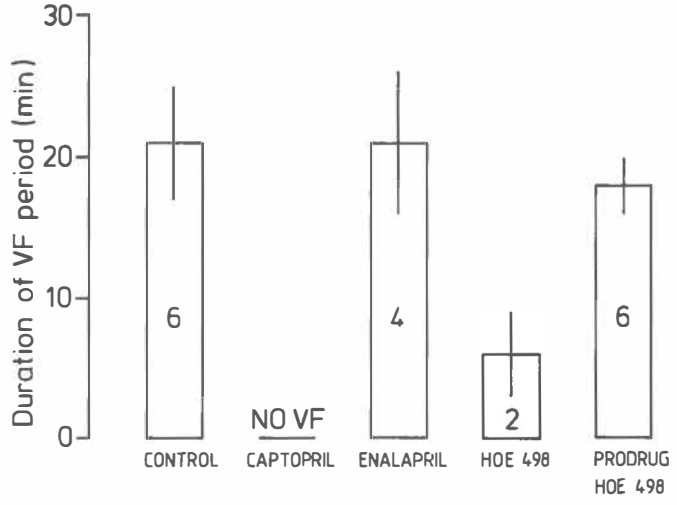

Figure 1. The incidence and duration (min) of ventricular fibrillation (VF) on reperfusion for treated and untreated hearts are indicated. The values in the vertical bars represent the total number of hearts that fibrillated upon reperfusion in each group (n=6).
*Significantly shorter duration of VF when compared with the control group ($p < 0.05$).

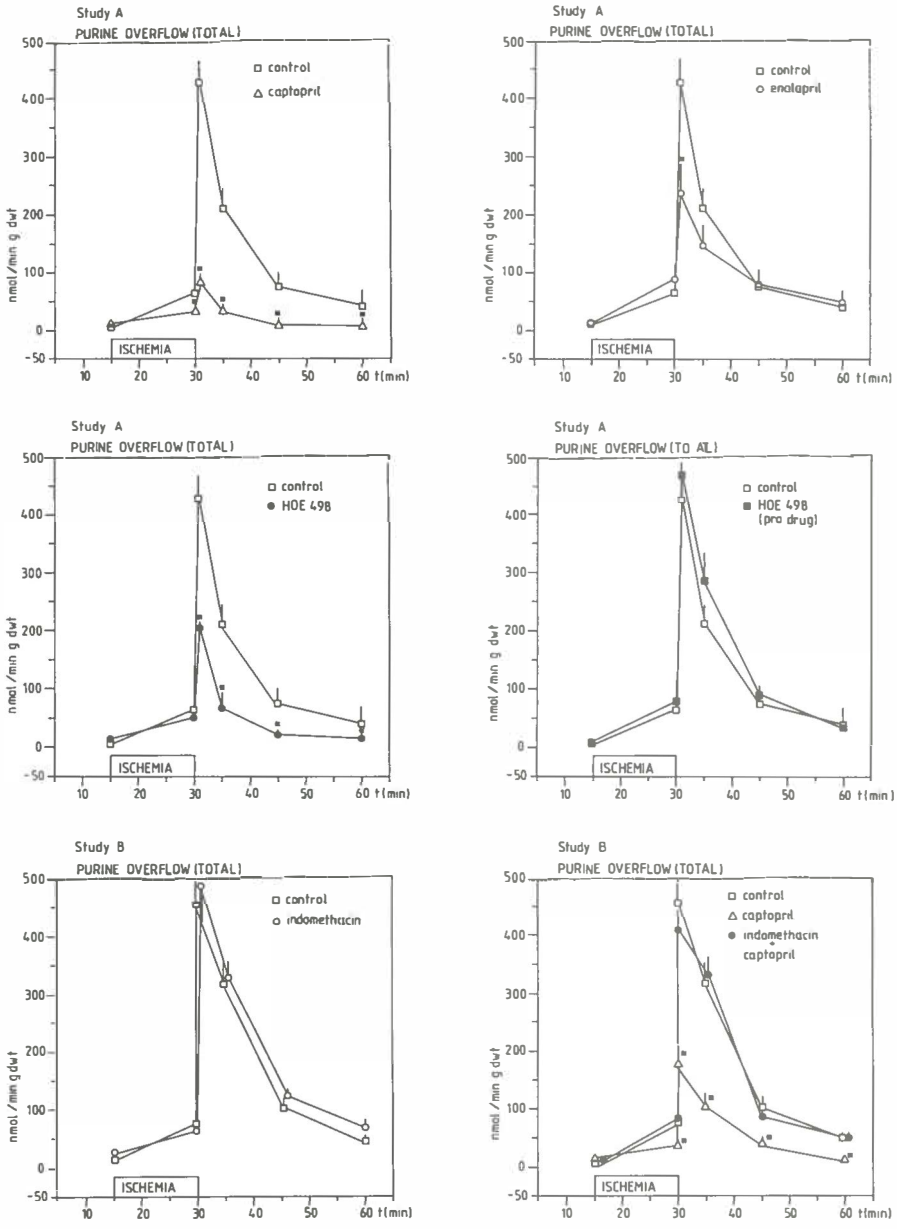

Figure 2. The total overflow of purine nucleosides and oxypurines during the experiment for treated and untreated hearts are plotted against time (minutes, abscissa). The ischemic period is indicated by an open bar on the abscissa. The measured values are calculated as nanomoles per minute and are expressed per gram dry weight (g dwt). Symbols are mean ± SEM (n=6).
*Significanty lower overflow when compared with the untreated group ($p < 0.05$).

ter 30 minutes of reperfusion, the pressure-rate index in the captopril and HOE- 498 treated group had recovered to values that did not differ significantly from the initial values ($p < 0.001$ for both groups when compared to the untreated group). This effect of captopril was also abolished by simultaneous addition of indomethacine (Table I, study B).

Upon reperfusion, a massive overflow of purines was detected in all groups (Figure 2). This purine overflow was significantly reduced by captopril and HOE 498 treatment during the whole reperfusion period. Enalapril treatment

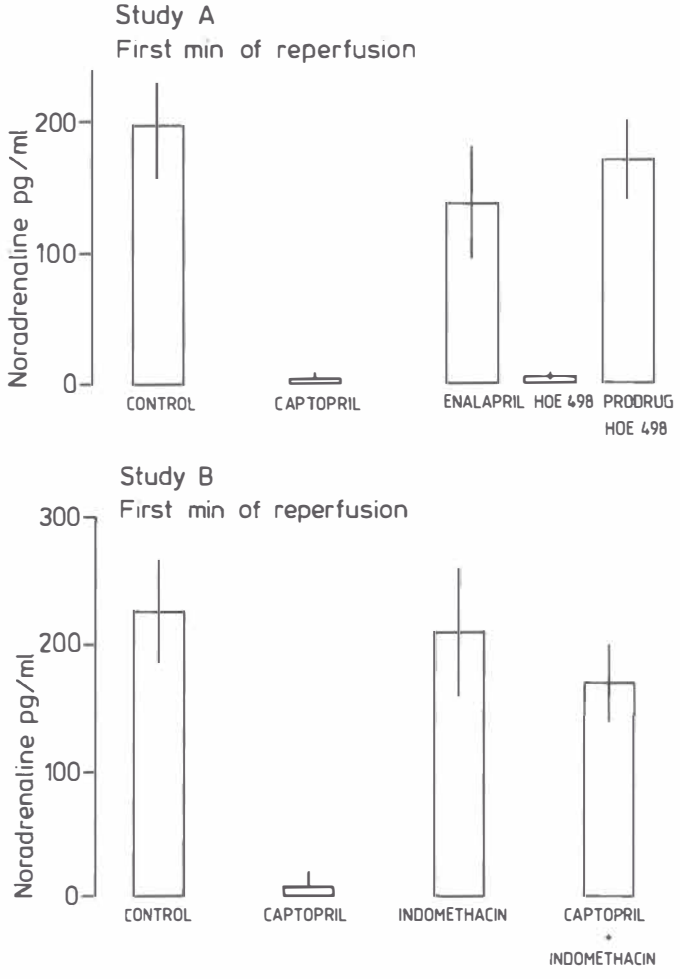

Figure 3. The concentration of noradrenaline in the coronary effluent of treated and untreated hearts during the first minute of reperfusion is indicated.
*Significant lower concentration of noradrenaline when compared with the untreated group ($p < 0.05$).

resulted in a significant reduction of purine overflow during the first minute of reperfusion only. Prodrug HOE 498 had no significant effect on purine overflow when compared to the untreated group (Figure 2, study A).

Upon reperfusion, a sharp rise in noradrenaline concentration in the coronary effluent was detected in the untreated group (Figure 3A and B). A similar release of noradrenaline was seen in the enalapril and prodrug HOE 498 groups. In contrast, noradrenaline concentrations in the coronary effluent of the captopril and HOE 498 group were below detection level (5 pg/ml) during the first minute of reperfusion (Figure 3, study A). Indomethacine abolished these effects of captopril, both on purine overflow (Figure 2, study B) and on noradrenaline overflow (Figure 3, study B). During reperfusion, the angiotensin II levels remained below detection level (2 pg/ml) in untreated hearts.

Discussion

In this study we compared the angiotensin converting enzyme inhibitors captopril, enalapril, and HOE 498 at concentrations that are equipotent in their effect on angiotensin I pressor response. [21,22] Their effects on the incidence and duration of ventricular fibrillation caused by reperfusion after 15 minutes of local ischemia produced by coronary artery ligation in the isolated rat hearts were investigated. Furthermore, the influence of cyclooxygenase inhibition on the protective effects of captopril was studied. Earlier results have shown beneficial effects of captopril in this model. [7,23] These findings were confirmed in this study. HOE 498 also appeared to be effective in this model, although its beneficial effects were limited to the active, angiotensin converting enzyme inhibiting form. No significant effects were observed for the prodrug. In contrast, enalapril did not influence arrhythmias and hemodynamics during reperfusion significantly, suggesting that the mechanism is independent of the inhibition of angiotensin converting enzyme. However, enalapril did reduce purine overflow significantly during the first minute of reperfusion. Therefore, it can not be excluded that other factors such as the concentration used and time to peak effect, contribute to the absence of a beneficial effect of enalapril in this model.

A striking finding in the present study was the apparent association of the protection by captopril and HOE 498 against reperfusion arrhythmias and the abolishment of noradrenaline overflow upon reperfusion. If there is a causal relationship between these events, the mechanism of the noradrenaline release and its possible modulation by converting enzyme inhibitors are important to elucidate. Local factors that might mediate the noradrenaline release observed in the control group include extracellular hyperkalemia, which depolarizes the nerve endings. [24] Acidosis and hypoxia have also been implicated in

the direct, i.e., nerve impulse independent, increase in noradrenaline release from the ischemic heart. The formation of membrane-active metabolites, such as lysophosphoglycerides, in ischemic tissue may also increase noradrenaline release secondary to depolarization of the nerve ending with concomitant influx of calcium. [25] A reduced reuptake of noradrenaline has not been proposed as a major cause of depletion of myocardial stores. However, reuptake is an active process that will be impaired if there is an insufficient supply of high energy substrates during ischemia; [26] the latter is suggested by the massive overflow of ATP catabolites observed in this study in the control group. Therefore, impairment of noradrenaline reuptake still may play role in this noradrenaline overflow.

As captopril and HOE 498 reduce the vascular overflow of noradrenaline upon reperfusion, they seem to interfere with the neurogenic noradrenaline release from the presynaptic sympathetic nerve endings and not directly with the postsynaptic myocardial cells, as suggested earlier. [7] Several mechanisms may be involved in this effect of captopril and HOE 498 on catecholamine release. They might act directly on presynaptic receptors that are known to have a regulatory function in the local regulation of neurotransmitter release. However, at present, only postsynaptic antiadrenergic effects of captopril have been reported. [8]

Indirectly, a stimulation of prostaglandin synthesis may be involved, which is supported by our findings in study B. Indomethacin abolished all the effects of captopril in our reperfusion model; reperfusion arrhythmias, purine and catecholamine overflow were again comparable to untreated hearts. There is good evidence in vivo that enhanced prostacyclin production reduces reperfusion-induced arrhythmias. [27] Various other studies have pointed to prostaglandins as possible mediators of the action of converting enzyme inhibitors, [28,29] and recently, a direct stimulatory action of captopril on prostacyclin (PGI2) synthesis in vascular tissue was shown. [9] It is also known that PGI2 inhibits noradrenaline release caused by nerve terminal depolarization in the isolated rat and rabbit heart. [30] Thus, captopril and HOE 498 may reduce the catecholamine overflow indirectly via a stimulation of PGI2 synthesis. The results of our study B indicate that this mechanism plays an important role, at least for captopril, in this model, as indomethacin abolishes the effect of captopril on catecholamine overflow.

Although it is known that angiotensin II facilitates adrenergic transmission by enhancing noradrenaline release upon nerve depolarization, it is unlikely that a reduction of angiotensin II concentration is involved in the observed beneficial effects. No angiotensin II appears to be produced in the isolated perfused rat heart, although the presence of enzymes of the renin-angiotensin system has been demonstrated. [31] In this study, no angiotensin II was detected in the coronary effluent of untreated hearts, neither during ischemia nor during

reperfusion, using a sensitive radioimmunoassay technique. Moreover, the lack of effect of enalapril suggests that angiotensin converting enzyme inhibition is not involved in the reduction of catecholamine overflow upon reperfusion.

In conclusion, this study shows that captopril and HOE 498 reduce the incidence and duration of malignant reperfusion arrhythmias after coronary artery occlusion in the isolated rat heart. This reduction of reperfusion arrhythmias is probably angiotensin II independent and is associated with a reduction of noradrenaline overflow. It is suggested that facilitation of prostacyclin synthesis plays an essential role in the protective effects, as indomethacin abolishes these beneficial effects of captopril.

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APPENDIX 5

CONCENTRATION-DEPENDENT PROTECTION BY CAPTOPRIL AGAINST MYOCARDIAL DAMAGE DURING ISCHEMIA AND REPERFUSION IN A CLOSED-CHEST PIG MODEL

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Summary

We previously reported concentration-dependent protection of captopril against ischemia-reperfusion injury in the isolated rat heart. In order to study these effects in vivo, we developed a closed-chest pig model. Reversible occlusion of the left coronary artery was achieved with a PTCA catheter during one hour. Captopril was given intravenously in two different concentrations (0.6 mg/kg/10 minutes ± 0.3 mg/kg/2 hours and 6 mg/kg/10 minutes ± 3.0 mg/kg/2 hours) during the experiments to 11 and 10 pigs, respectively, versus 12 controls, who received only saline. Due to malignant ventricular arrhythmias, nine pigs died during ischemia. At the end of the reperfusion period of 2 hours, eight pigs were alive in each group. In the captopril-treated pigs, maximum creatine kinase after two hours of reperfusion was significantly lowered to 6,337 ± 709 U/liter in the high-dose group versus 8,285 ± 851 U/liter in the low-dose group and 9,635 ± 1,115 U/liter in the saline group. A reduction of local inosine overflow in the coronary sinus was seen. Maximum noradrenaline overflow after 5 minutes reperfusion diminished dose-dependently to 695 ± 284 and 3,129 ± 1,728 pg/ml in the captopril-treated groups versus 4,693 ± 2,277 pg/ml in the saline-treated group. Mean arterial blood pressure and cardiac output decreased significantly during ischemia and reperfusion, but no significant differences occurred between the treated and untreated groups. Reperfusion arrhythmias, mainly accelerated idioventricular rhythm disturbances, were comparable among the three groups.

We conclude that in vivo administration of captopril reduces myocardial damage upon reperfusion after one hour of ischemia in a dose-dependent way. It is suggested that direct myocardial effects play an important role in the underlying mechanism.

Introduction

The effects of angiotensin converting enzyme inhibitors on limiting infarct size after coronary occlusion remain controversial. [1-5] Earlier reported results of a beneficial effect of captopril on infarct size [1] were not found by others. [2,3] Studies on cardioprotective effects of another angiotensin converting enzyme inhibitor, enalapril, were less ambiguous, [4,5] but these data await further corroboration by other investigators. The reasons for these conflicting results remain unclear, but differences in animal species, duration of ischemia, concentration, and type of the angiotensin converting enzyme inhibitor used may all be contributing factors.

We recently described concentration-dependent protection by captopril against reperfusion injury in the isolated rat heart. [6,7] In this study, captopril markedly reduced the incidence and duration of ventricular fibrillation upon reperfusion after 15 minutes of local ischemia produced by coronary artery ligation. As was shown by the reduced purine overflow in those experiments, myocardial cells were protected against reperfusion damage.

The aim of this study was to investigate whether these beneficial effects of captopril against ischemia-reperfusion injury are also present in vivo. To realize this, we developed a closed-chest pig model, in which reversible occlusion of the left coronary artery was achieved with a balloon catheter during one hour.

Methods

Preparation

Male Yorkshire swine (body weight 25-35 kg) were pretreated with 120 mg azaperone (Stresnil; Janssen Pharmaceutica Beerse, Belgium) intramuscularly. Half an hour later, 150 mg metomidate (Hypnodil; Janssen) was injected in an ear vein. A cuffed endotracheal tube was introduced, and the animals were ventilated with a mixture of O_2/N_2O. Anesthesia was maintained with an intravenous infusion of azaperone (2 mg/kg/minute) and metomidate (8 mg/kg/minute) through a double-lumen catheter in the inferior vena cava. Ventilation parameters were adjusted to keep arterial pCO_2 concentrations between 4.5 and 6.5 kPa and pO_2 concentrations between 16 and 20 kPa. Body temperature

was kept at 36-38°C with a thermal matress. Heparin was administered at an initial dose of 5,000 IU, followed by 2,500 IU/h.

Acute regional myocardial ischemia was produced with a balloon catheter as used for percutaneous coronary angioplasty (PTCA). A modified no. 7 French Sones catheter was introduced via the left carotid artery and the tip was placed at the ostium of the left coronary artery as verified by fluoroscopy. Through the lumen of this catheter, the PTCA catheter was positioned in the anterior descending branch of the left coronary artery and advanced beyond the first diagonal artery. In order to obtain reproducible ischemic regions, the balloon was carefully checked by contrast fluoroscopy.

Protocol

The animals were allowed to equilibrate until stable baseline values were observed. After this equilibration period and a control period of 30 minutes, the PTCA catheter was introduced, and ischemia was induced by inflating the balloon. After 60 minutes ischemia, the balloon was deflated and the catheter removed. After reperfusion of the ischemic zone for 120 minutes, the experiments were terminated and the pigs subjected to routine postoperative care.

Captopril was given intravenously in two different concentrations. One group of 11 pigs was infused during the first 10 minutes of the control period with 0.6 mg/kg captopril and received 0.0025 mg/kg/minute during the next 120 minutes. Another group of 10 pigs was treated with a bolus infusion of 6.0 mg/kg captopril, followed by continuous infusion of 0.025 mg/kg/minute during the next two hours. A group of 12 pigs received only saline and served as control. In order to prevent lethal ventricular arrhythmias during the early phase of ischemia, a bolus injection of lidocaine (1 mg/kg intravenously) was administered to all animals two minutes before ischemia. When ventricular tachycardia/fibrillation occurred, direct current cardioversion was applied.

Hemodynamics

Throughout the experiments, arterial blood pressure was measured by a catheter in the left femoral artery. Heart rate and arrhythmias were monitored continuously by electrocardiography. A triple-lumen Swan-Ganz catheter was inserted via the external jugular vein and placed in position in order to determine right atrial pressure and cardiac output. Stroke volume and systemic vascular resistance were calculated from these data.

Biochemical assays

In order to quantify myocardial damage during ischemia, we collected blood samples for monitoring creatine kinase levels at the end of the ischemic period and during reperfusion at 5, 30, 90 and 120 minutes. Serial changes in plasma

creatine kinase levels have been widely used to estimate myocardial infarct size in experimental models, and there appears to be good agreement between the area under the creatine kinase curve and infarct weight as determined at autopsy. [3,8]

Moreover, we measured the ATP catabolite inosine in the coronary effluent at the end the control period and of the ischemic period and during reperfusion at 1, 5, 15 and 30 minutes. For this purpose, a polyethylene catheter was introduced in the coronary sinus via the left coronary vein. Local ATP catabolites may reflect myocardial ATP breakdown and can be used as a biochemical marker for the extent of ischemia. [9] The samples were also assayed for noradrenaline levels by a sensitive HPLC method with electrochemical detection in order to quantify overflow of this catecholamine. [10] Reperfusion of ischemic myocardium can be accompanied by a sudden increase in noradrenaline overflow, [11], which may contribute to the severity of reperfusion damage.

Statistical analysis

Statistical analysis was performed with Student t-test or the Wilcoxon rank sum test if the assumption of a normal distribution had to be rejected. Differences were considered to be significant at a p value less than 0.05, two-sided. Results are given as the mean values ± SEM.

Results

Arrhythmias

During the control period, no serious arrhythmias were noted. After ischemia was induced, in most animals malignant ventricular rhythm disturbances developed especially during the first five minutes and between 20 and 30 minutes. In the saline-treated group and in the low-dose captopril group, ventricular tachycardia/fibrillation occurred in eight animals of each group and in the high-dose captopril group in 5 animals. Direct current cardioversion was unsuccessfull in 4, 3 and 2 pigs, respectively, and at the end of the ischemic period eight pigs were alive in each group. There were no significant differences in the incidence and duration of ventricular arrhythmias during ischemia among the three groups.

Upon reperfusion, no ventricular fibrillation occurred. After two minutes, all but two pigs showed a continuous or intermittent accelerated idioventricular rhythm in a range of 100 to 160 bpm. Total duration during the first 30 minutes was 8.9 ± 3.3 minutes in the saline-treated group versus 11.7 ± 3.4 minutes and 12.5 ± 4.8 minutes in the low and high-dose captopril groups respectively (n.s.). Reperfusion arrhythmias were comparable among the groups.

Hemodynamics

Baseline values of stroke volume and mean arterial blood pressure were comparable between all groups. After the bolus injection of captopril, a small, but significant, decrease in mean arterial blood pressure was noted, but at the end of the control period no significant differences were present. This correlated with low levels of plasma renin activity, even in the captopril-treated groups (results are not shown). Alterations of the hemodynamic parameters during ischemia and reperfusion are shown in Table 1. After ischemia was induced, cardiac output and stroke volume decreased significantly with a marked drop in blood pressure and an increased heart rate.

Further deterioration occurred during the reperfusion period with persistent low mean arterial blood pressure and increase in heart rate. Except for a significantly lower mean arterial blood pressure in the low-dose captopril group at the end of the ischemic period, no significant differences were noted among the three groups. Systemic vascular resistance did not increase significantly during ischemia and reperfusion, except for the high-dose captopril group at the end of the reperfusion period.

Myocardial damage

Creatine kinase levels started to increase in each group five minutes after reperfusion. A dose-dependent reduction was present in the captopril-treated groups, which was significant at all time points in the high-dose captopril group (Figure 1A). The same differences were demonstrated by plotting the creatine kinase time course of each pig and determining the area under the curve, i.e., the total release of creatine kinase, from the end of the control period to the end of the reperfusion period (Figure 1B). Inosine levels were lower in the captopril-treated groups during ischemia, and the increase in overflow following reperfusion was markedly attenuated (Figure 2). This was statistically significant at 5 minutes of reperfusion and thereafter, without significant differences between the two groups.

Upon reperfusion, a substantial rise in noradrenaline levels in the coronary sinus blood was detected in the saline-treated group with a maximum after 5 minutes followed by a decline (Figure 3). This increase in noradrenaline was reduced in the captopril-treated groups in a dose-dependent way, reaching statistical significance in the high- dose captopril group.

Table 1. *Effects of captopril on heart rate and other hemodynamic parameters during ischemia and reperfusion.*

		0.6 mg/kg/10 min + 0.3 mg/kg/2 hr	*6.0 mg/kg/10min* ± 3.0 mg/kg/2 hr
	SALINE	*CAPTOPRIL*	*CAPTOPRIL*
		End control period (t=30)	
Cardiac output	103 ± 3	109 ± 4	103 ± 3
Stroke volume	101 ± 3	104 ± 5	101 ± 5
Mean arterial pressure	95 ± 2	98 ± 2	93 ± 2
Syst. vascular resistance	92 ± 3	90 ± 4	89 ± 5
Heart rate	102 ± 2	105 ± 2	103 ± 2
		End ischemia (t=90)	
Cardiac output	80 ± 4**	80 ± 5**	87 ± 8
Stroke volume	66 ± 4**	64 ± 5**	75 ± 6**
Mean arterial pressure	84 ± 3*	70 ± 4**†	84 ± 4
Syst. vascular resistance	104 ± 7	84 ± 7	98 ± 10
Heart rate	120 ± 6*	127 ± 13	114 ± 4*
		End reperfusion (t=210)	
Cardiac output	78 ± 5**	72 ± 7**	76 ± 3**
Stroke volume	64 ± 6**	52 ± 5**	56 ± 6**
Mean arterial pressure	82 ± 4*	71 ± 4**	86 ± 4
Syst. vascular resistance	105 ± 8	97 ± 9	110 ± 3*
Heart rate	124 ± 10	140 ± 16	140 ± 14*

Values are means ± SEM, expressed as percentage of the values at t = 0
* $p < 0.05$; ** $p < 0.01$ as compared with the control period
† $p < 0.05$ as compared with the saline group.

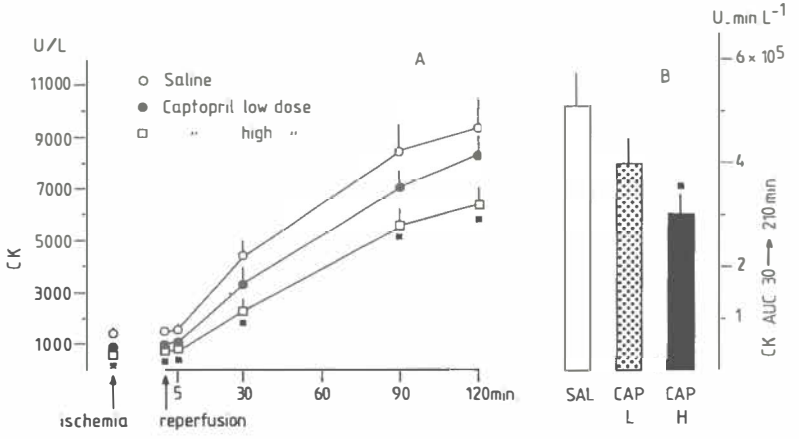

Figure 1. A: Serial changes of creatine kinase (CK) during ischemia and reperfusion in the saline- and captopril-treated groups. **B.** Total release of creatine kinase during ischemia and reperfusion (30 → 210 minutes) in the treated and untreated animals. CAP = Captopril; H = High-dose; L = Low-dose; SAL = Saline-treated group. Values are expressed as mean ± SEM. *$p < 0.05$.

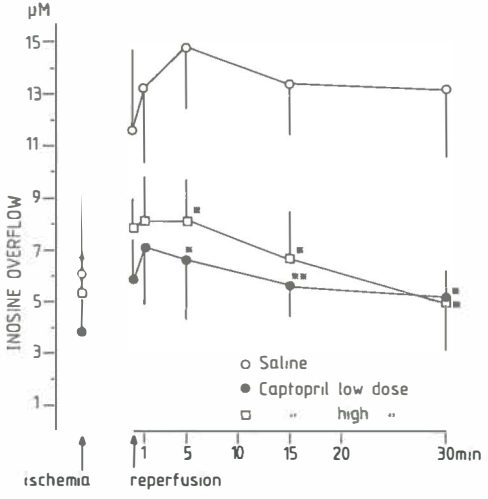

Figure 2. Time course of inosine overflow in the coronary sinus during ischemia and reperfusion in the saline- and captopril-treated groups. Values are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$.

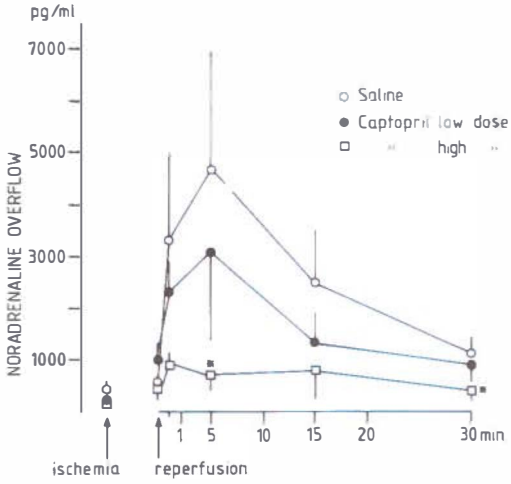

Figure 3. Time course of the noradrenaline overflow in the coronary sinus during ischemia and reperfusion in the saline- and captopril-treated groups. Values are mean ±SEM. *$p < 0.05$.

Discussion

Our in vivo results show that captopril reduces myocardial damage during reperfusion after one hour of ischemia in a dose-dependent way. This was demonstrated by a reduction in total creatine kinase release during ischemia and reperfusion. Although creatine kinase release is flow-dependent and thus subject to considerable variation, this is not the case after early reperfusion. [12] Thus, serial creatine kinase levels reliably reflect the size of myocardial damage under these circumstances. Concomitantly, a reduction in the overflow of inosine, an ATP catabolite, was detected, indicating a lesser degree of cellular damage during ischemia. [9]

In contrast to our studies in the isolated rat heart after 15 minutes of local ischemia, [6,7] no effect of captopril was seen on reperfusion arrhythmias in this pig model. A delayed accelerated idioventricular rhythm occurred in almost all pigs without significant differences between treated and untreated hearts. In the isolated rat hearts, all untreated hearts showed ventricular fibrillation immediately after reperfusion with a dose-dependent reduction in the incidence and duration when captopril was added. These two distinct types of reperfusion arrhythmias have been demonstrated earlier, and the underlying mechanisms are probably different. [13] A critical factor in this regard is the duration of ischemia. It has been suggested that the decline in vulnerability to ventricular fibrillation is due to the onset of irreversible cellular injury. [14] Irreversible myocardial injury has been reported to occur between 15 and 30 minu-

tes of ischemia. [15] Therefore, cellular damage in the rat heart was mainly reversible, leading to an increased sensitivity for ventricular fibrillation, but not in the pig because ischemia was prolonged (60 minutes).

Earlier studies with captopril on the effects on infarct size gave conflicting results. [1-3] These studies were performed in dogs. We chose the pig model, because the pig heart, in contrast to the dog, lacks significant collateral blood flow, and the pig coronary vascular system is more closely related to that of man. [16] The investigators only looked at the effects during ischemia and did not take into account the possible deleterious effects which can occur following reperfusion. [17] This may be important in the clinical situation since early coronary artery recanalisation can occur spontaneously in a considerable number of patients with acute myocardial infarction. [18] Moreover, with the increasing use of thrombolytic therapy, attention will be more and more focused on the prevention of reperfusion damage. However, it must be emphasized that high concentrations of captopril were used in our study.

The underlying mechanisms of these cardioprotective effects of captopril remain to be established. Several mechanisms can be postulated. The most obvious one is a reduction of myocardial oxygen consumption due to a decrease in pre- and afterload. Remarkably, however, was the lack of effect of captopril on the altered hemodynamics associated with ischemic myocardial damage, despite the fact that significant changes in mean arterial blood pressure and cardiac output occurred. This had been demonstrated previously. [3] Therefore, other factors may be important, which involve the heart itself.

In the first place this may include an increase in coronary blood flow to the ischemic region, as has been shown by other investigators. [1,4] This may be mediated by inhibition of the local vasoconstrictor effects of angiotensin II or by potentiation of bradykinin and its vasodilating effect. [5] We have demonstrated a direct vasodilating effect of captopril in vitro, which may be due to interference with the arachidonic acid metabolism. [19] Second, inhibition of the sympathetic nervous system, which is normally activated following acute myocardial infarction, [20] may play a role. In our study, a reduction in the overflow of noradrenaline after reperfusion was seen in the captopril-treated groups. However, this did not result in a reduction in heart rate or in incidence of reperfusion arrhythmias. This may indicate that local processes, such as damaged nerve endings, are involved, which are prevented by captopril. Third, one might speculate whether the efficacy of captopril in preventing postischemic tissue injury is at least partly due to its potential as a scavenger of free radicals, since captopril possesses a sulfhydryl group. Generation of oxygen-derived free radicals can play an important role in the pathogenesis of cardiac ischemic injury and the exacerbation by reperfusion. [21] However, cardioprotective effects have been noted with another, non-sulfhydryl angiotensin converting enzyme inhibitor, [4,5] and so far little is known about the role of captopril as a free

radical scavenger. This also applies to the lysosomal membrane-stabilizing activity, which has been described with captopril, but not with enalapril. [4]

In summary, we have demonstrated that captopril in vivo is able to reduce myocardial damage during reperfusion after one hour of ischemia. This effect is dose-related and most pronounced at high concentrations. Clinical studies in the situation of impending myocardial infarction, for instance after thrombolytic therapy, will have to determine whether these results can be extrapolated to the human situation.

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APPENDIX 6

PROTECTIVE EFFECTS OF CAPTOPRIL AGAINST ISCHEMIA/REPERFUSION-INDUCED VENTRICULAR ARRHYTHMIAS IN VITRO AND IN VIVO.

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Summary

The effects of the converting enzyme inhibitor captopril on the susceptibility of the heart to ventricular arrhythmias following ischemia, both in vitro and in vivo, were studied. In isolated rat hearts, captopril, administered either before or at the end of ischemia, reduced ventricular fibrillation upon reperfusion after 15 minutes of local ischemia. Reduction of purine overflow, improvement in contractility, and increase in coronary blood flow occurred concomitantly. In vivo, a closed-chest pig model was used to determine the effects of captopril, administered at the end of ischemia and continued orally, on the susceptibility to ventricular arrhythmias during the chronic phase of myocardial infarction. Myocardial ischemia was induced by 60-minute inflation of a balloon catheter in the left anterior descending coronary artery. Upon reperfusion, an accelerated idioventricular rhythm occurred, both in 10 untreated and in 10 captopril-treated animals. Creatine kinase levels during the reperfusion period were significantly lower after captopril treatment. Two weeks after the short-term experiments, monomorphic ventricular tachycardia could be induced with programmed electrical stimulation in six of eight surviving untreated pigs. In contrast, in none of the six surviving captopril-treated animals ventricular tachycardia was inducible.

Thus, early intervention with captopril during the development phase of myocardial infarction may have beneficial effects on the subsequent development of ventricular arrhythmias. Salvage of ischemic myocardium, improvement in ventricular function, beneficial effects on coronary flow, and decreased activity of the sympathetic nervous system may all contribute.

Introduction

Little is known about the role of the renin-angiotensin- aldosterone system, and of angiotensin II, in particular, in the pathogenesis of ventricular arrhythmias in ischemic heart disease. However, it has been suggested that angiotensin II may be detrimental through various mechanisms, including reduced coronary blood flow, increased cardiac afterload, sympathetic activation, and decreased total body potassium. [1-6] In addition to the plasma renin-angiotensin-aldosterone system, the local renin-angiotensin-aldosterone system may contribute to these effects. [7,8]

If an arrhythmogenic effect of angiotensin II really exists, then inhibition of the angiotensin converting enzyme, either local or systemic, should result in a reduction of ventricular arrhythmias in ischemic heart disease. This hypothesis is supported by an earlier study demonstrating that intravenous captopril reduced the inducibility of sustained ventricular tachycardia one week after experimental myocardial infarction in pigs. [9] Moreover, clinical trials with captopril [3] and enalapril [4,5] in patients with congestive heart failure have shown a reduction in the frequency and complexity of ventricular arrhythmias compared with results of placebo treatment. However, angiotensin-independent mechanisms may also contribute to some of the antiarrhythmic effects. We have shown that facilitation of prostacyclin synthesis by a reduction of bradykinin breakdown plays an important role in the protective effects of captopril against reperfusion arrhythmias in isolated rat hearts. [10,11] Since activation of the renin-angiotensin- aldosterone-system occurs during hypoxia, [12] an important question is whether or not inhibition of this system following ischemia will lead to subsequent reduction in ventricular arrhythmias. This was studied in two animal models, both in vitro and in vivo.

Methods

Isolated rat heart model

Preparation of the heart

Male Wistar rates (275-325 g), fed ad libitum, were anesthetized with ether and given 5,00 IU of heparin intravenously. The hearts were rapidly excised and arrested in ice-cold 0.9% NaCl. Retrograde perfusion of the aorta, as described by Langendorff, was immediately started using a modified Tyrode solution. [9] The hearts beat spontaneously. The temperature was kept between 36.5 and 37.5 °C.

Acute regional myocardial ischemia was produced by occlusion of the left coronary artery. This was accomplished by tightening a previously placed liga-

ture round the descending branch 2 mm below the aortic root. The myocardium was reperfused by releasing this ligature.

Measurement of mechanical and electrophysiological parameters

Left ventricular end-systolic pressure was measured by means of a catheter inserted into the left ventricle via the left atrium and the mitral valve and connected to a pressure transducer. A bipolar cardiac electrogram was obtained by means of two electrodes: one attached to the metal inflow cannula and the other to the ventricular apex outside the ischemic zone. Heart rate and the occurrence of arrhythmias were monitored by continuous registration of the cardiac electrogram. The electrocardiographic recording was made with an ink jet recorder at a paper speed of 25 mm/second. Coronary flow (volume of perfusion fluid per time unit) was measured by a microprocessor, which controlled the perfusion pressure by adjusting the peristaltic pump. In the coronary effluent, the total overflow of the purine adenosine and its catabolites (inosine, hypoxanthine, and xanthine) was measured as an indicator of nucleotide breakdown in the tissue. This has proved to be a reliable parameter for the quantitative assessment of ischemia-induced cellular damage. [13]

Protocol

The hearts were allowed to equilibrate with the perfusion fluid for 15 minutes. After this equilibration period and a control perfusion of 15 minutes, local ischemia was induced and maintained for the next 15 minutes. After reperfusion of the ischemic zone for 30 minutes, the experiments were terminated.

The rat hearts were divided at random into three groups of six each. In one group, captopril was added to the perfusate at the start of the control period in a concentration of 80 μg/ml, and treatment was continued during the entire experiment. In the second group, captopril was given in the same concentration at the end of the ischemic period and continued during the reperfusion period. One group served as the control group. Fresh solutions were prepared daily.

Closed-Chest Pig Model

Acute-phase experiments

Male Yorkshire swine (body weight, 25-35 kg) were anesthetized, as described before. [14] Body temperature was kept at 36-38°C with a thermal matress. Heparin was administered at an initial dose of 5,000 IU, followed by 2,500 IU/hour. Arterial blood pressure was monitored with a catheter in a femoral artery. Blood samples taken from this catheter were used for monitoring creatine kinase levels in order to quantify irreversible myocardial damage. [15] Cardiac output was monitored by the thermodilution method with a Swan-Ganz cathe-

ter advanced through the external jugular vein. A polyethylene catheter was introduced into the coronary sinus via the same vein. Blood samples were drawn for the determination of the norepinephrine overflow, [15] plasma renin activity, [16] and inosine concentration [13] from the ischemic and reperfused myocardium. Acute regional myocardial ischemia was produced with a balloon catheter as used for percutaneous coronary angioplasty (PTCA). This catheter was introduced through the lumen of a modified 7 French Judkins catheter via the left carotid artery and positioned under radiographic control in the anterior descending branch of the left coronary artery, beyond the first diagonal side branch. Following positioning of the catheters, an equilibration period was allowed until stable baseline values were observed. After this equilibration period and a control period of 30 minutes, ischemia was induced by inflating the balloon for 60 minutes. After reperfusion was achieved by deflation of the balloon, the animals were observed for another 120 minutes. Subsequently, the experiments were terminated. After removal of the catheters, pigs were subjected to postoperative care and returned to their cages. The study included 24 pigs. During ischemia, four pigs died due to ventricular fibrillation. At the end of ischemia, the animals were randomly divided into two groups of 10 each. In one group a bolus injection of 6 mg/kg captopril was given intravenously prior to reperfusion. The other group received intravenous saline solution only and served as a control group.

Programmed electrical stimulation two weeks after the acute-phase experiment

Captopril was continued in an oral dose of 150 mg daily in the animals that had received an intravenous bolus injection during the acute phase of the experiment. The saline-treated pigs received routine care only. Two weeks after the occlusion/reperfusion procedure, programmed electrical stimulation of the heart was performed in the surviving animals, to evaluate the inducibility of ventricular tachycardia or fibrillation. Induction and maintenance of anesthesia were the same as described before. Positioning of electrode catheters and stimulation protocol were performed as described earlier. [17] Stimuli were delivered at two times the diastolic threshold. The stimulation protocol included stimuli of the right ventricular apex or right ventricular outflow tract during sinus rhythm and at three different cycle lengths of 500, 400, and 300 msec using single, double, and triple stimuli. Four surface (Einthoven I, II, III and a precordial lead) electrocardiograms and three intracardiac electrograms (HRA, HBE, and RVA) were recorded on magnetic tape and registered on an ink jet recorder at a paper speed of 100 mm/second. The His bundle recordings were used to discriminate intraventricular reentrant beats from nonstimulated beats caused by bundle branch reentry. Animals were regarded as demonstra-

ting inducibility when ventricular tachycardia or ventricular fibrillation was reproducibly initiated during programmed electrical stimulation. Tachycardia was defined as sustained when it lasted more than 30 seconds and as nonsustained if there were at least six nonstimulated beats. Induced ventricular tachycardia was terminated by overdrive pacing, occasionally by direct current counter shock. In the animals of the control group, which demonstrated inducibility, the same protocol was repeated five minutes after administration of a bolus injection of captopril (0.6 mg/kg of body weight intravenously). If this dose failed to prevent the initiation of ventricular tachycardia, the dose was repeated once.

Statistical analysis

The data are expressed as the mean ± SEM. Statistical analysis was performed with Student t test, Fisher exact test or the Mann Whitney U test if the assumption of a normal distribution had to be rejected. Differences were considered significant at a p value of less than 0.05 (two-sided).

Results

Isolated rat heart model

Hemodynamic changes

At the beginning of the control period, no significant differences in coronary flow were present among the three groups: 9.9 ± 0.8 ml/minute for control hearts, 11.4 ± 0.5 ml/minute for hearts treated with captopril at the end of the ischemic period, and 11.3 ± 0.3 ml/minute for hearts treated with captopril during the entire experiment. Ligation of the left coronary artery resulted in reductions of coronary flow comparable to those at the end of the control period (58, 63, and 66 percent, respectively). When captopril was added to the perfusate during the entire experiment, a persistent and significant increase in coronary flow was seen, already present during the control period. Correspondingly, when captopril was given at the end of the ischemic period, an increase in coronary flow occurred during reperfusion, significant after five minutes and still present after 30 minutes of reperfusion (Table 1). Changes in left ventricular pressure demonstrated a significant increase during the control period and less reduction during ischemia in the captopril-treated group in comparison with the two other groups. When captopril was administered at the end of the ischemic period, an improvement of this parameter of contractility occurred, although this effect was not significant (Table 1).

Table 1.

Effects of 80 μg/ml captopril on hemodynamic parameters during control perfusion, ischemia and reperfusion in isolated rat hearts (captopril was given during the entire experiment or only during reperfusion).

		CAPTOPRIL GROUP	
	CONTROL GROUP	Only During Reperfusion	During Entire Experiment
End of control period			
Coronary flow	90 ± 4	85 ± 2	130 ± 2‡
Heart rate	99 ± 3	98 ± 2	99 ± 2
Left ventricular pressure	110 ± 6	105 ± 3	145 ± 10*
End of ischemic period			
Coronary flow	52 ± 3	58 ± 3	93 ± 5‡
Heart rate	95 ± 1	91 ± 5	101 ± 4
Left ventricular pressure	26 ± 4	30 ± 5	44 ± 12
End of reperfusion period			
Coronary flow	87 ± 9	124 ± 5†	132 ± 4‡
Heart rate	97 ± 4	99 ± 4	99 ± 4
Left ventricular pressure	87 ± 17	129 ± 17	147 ± 17*

Values are mean ± SEM, expressed as percentage of the values at t=0.
*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$, compared with the control group.

Cellular injury

The purine overflow in the coronary effluent is shown in Figure 1. During the control period, low levels were present in all three groups. After coronary ligation, the concentration of purines increased slightly in all hearts. Upon reperfusion, the largest increase in purine overflow was seen in the untreated hearts (to 649 ± 76 nmol/minute/g of dry weight). This was significantly less in the groups that received captopril, not only when given from the control period onwards (to 210 ± 44.5 nmol/minute g of dry weight), but also, although less pronounced, when administered only during the reperfusion period (to 408 ± 47 nmol/minute/g of dry weight).

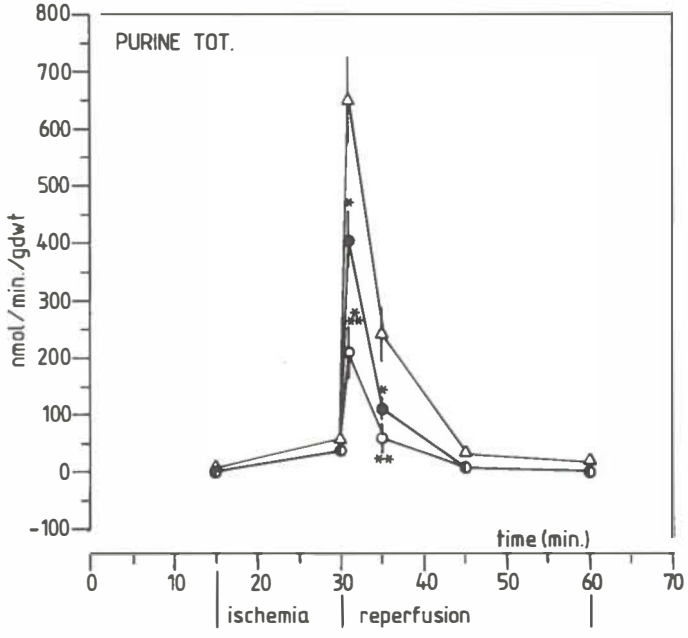

Figure 1. Time course of the purine overflow in the coronary effluent during ischemia and reperfusion in control hearts (△-△) and captopril-treated (80 μg/ml) hearts, given continuously (○-○) or only during reperfusion (●-●). The measured values are calculated as nmol/minute/ g of dry weight (gdwt). Values are mean ± SEM.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, compared with the control group.

Ventricular arrhythmias

During ischemia, infrequent ventricular premature beats occurred in all hearts, but no ventricular fibrillation was seen. Upon reperfusion, all untreated hearts, except one, showed ventricular fibrillation, with a mean duration of 92 ± 37 seconds. When captopril was present in the perfusate throughout

the experiment, ventricular fibrillation occurred in only two of the six hearts (duration 55 ± 32 seconds). When captopril was given during the reperfusion period only, ventricular fibrillation was seen in three of six hearts (duration 35 ± 15 seconds).

Closed chest pig model

Acute-phase experiments

Hemodynamic parameters

Baseline values of stroke volume, heart rate and mean arterial pressure were comparable between the two groups during the control period (Table 2). Similarly, changes during ischemia were comparable, with an increase in heart rate (significant only in the saline-treated group) and reductions in cardiac output and mean arterial pressure. Only a small decrease in blood pressure of short duration occurred when the bolus injection of captopril was given. During the reperfusion period, further deterioration of stroke volume and mean arterial blood pressure was noted, comparable in both groups. At the end of this period, heart rate was slightly higher in the saline-treated group. A marked increase in plasma renin activity was present during reperfusion (not different between the groups).

Myocardial damage

During ischemia, only a small increase in creatine kinase levels occurred (Figure 2). Following reperfusion, levels increased rapidly, with a maximum at the end of the reperfusion period (210 minutes). Creatine kinase levels were consistently lower in the captopril-treated group, reaching statistical significance 30 minutes following reperfusion and thereafter. Maximal inosine overflow after five minutes reperfusion decreased correspondingly to 5.9 ± 1.6 μM versus 8.4 ± 2.7 μM in the control group. During the first minute of reperfusion, a substantial increase in norepinephrine levels was detected in the coronary sinus blood, without significant differences between the groups.

Ventricular arrhythmias during reperfusion

Upon reperfusion all pigs demonstrated a continuous or intermittent accelerated idioventricular rhythm with a rate of 100 to 160 beats/minute. No ventricular fibrillation occurred, and no significant differences were noted between the captopril-treated group and the saline-treated group.

Table 2.

Changes in hemodynamic parameters and plasma renin activity during ischemia and reperfusion in pigs (6 mg/kg of captopril was given intravenously at the end of the ischemic period in 10 pigs, and a comparison was made with 10 saline-treated pigs).

	End of Control Period (30 minutes)		End of Ischemic Period (90 minutes)		End of Reperfusion Period (210 minutes)	
	Saline	Captopril	Saline	Captopril	Saline	Captopril
Mean arterial pressure (mmHg)	79 ± 3	76 ± 3	71 ± 2	66 ± 3*	66 ± 2†	64 ± 3*
Stroke volume (ml)	40 ± 3	36 ± 2	28 ± 2‡	26 ± 2‡	22 ± 2‡	22 ±2‡
Heart rate (beats/minute)	87 ± 5	88 ± 5	115 ± 8†	98 ± 4	117 ± 8†	108 ± 7*
Plasme renin activity (log nmol A1/liter/hr)	1.7 ± 0.4	3.2 ± 0.9	2.7 ± 0.7	3.7 ± 0.8	8.2 ± 4.9	10.9 ± 4.1

Values are mean ± SEM
*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$, compared with values at $t = 0$.

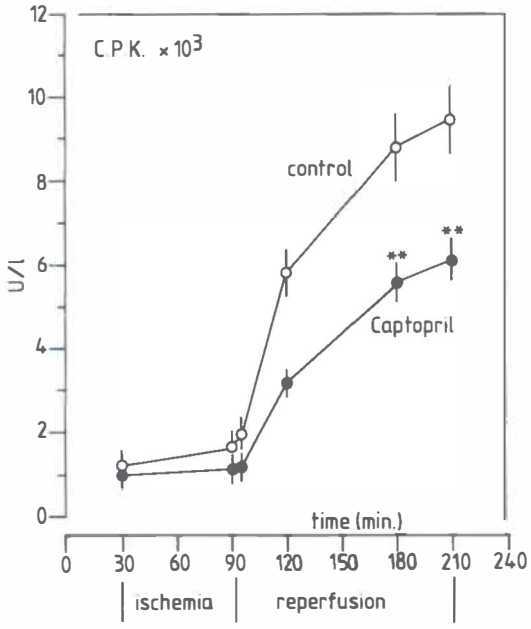

Figure 2. Time course of creatine kinase (C.P.K.) plasma levels during ischemia and reperfusion in control pigs and captopril-treated pigs. Captopril (6 mg/kg intravenously) was given as a bolus injection at the end of ischemia (t=90). Symbols represent mean ± SEM. $^{**}p < 0.01$.

Programmed electrical stimulation after two weeks

A flow chart of the successive phases is shown in Figure 3. Following the acute-phase experiments, four pigs in the captopril-treated group and two in the saline-treated group died within 24 hours. It was assumed that this was due to pump failure, but the cause of death remains unknown. After two weeks, programmed electrical stimulation was performed in the remaining 14 animals. At the beginning of these experiments, pressure-rate index was significantly higher in the control group (11,200) than in the captopril-treated group (8,300). In none of the six captopril-treated pigs was ventricular tachycardia, either sustained or nonsustained, inducible. In contrast, six out of eight control pigs showed ventricular tachycardia after programmed electrical stimulation, five with sustained monomorphic and one with nonsustained monomorphic ventricular tachycardia ($p < 0.05$ compared to the captopril-treated group). Maximal creatine kinase levels during the acute-phase experiments were 9,798 ± 1,249 units/liter in the inducible animals and 6,682 ± 890 in the pigs without inducible ventricular tachycardia ($p = 0.12$). After intravenous administration of captopril, sustained ventricular tachycardia could no longer be induced in two of the five animals that had previously demonstrated sustained monomorphic ventricular tachycardia.

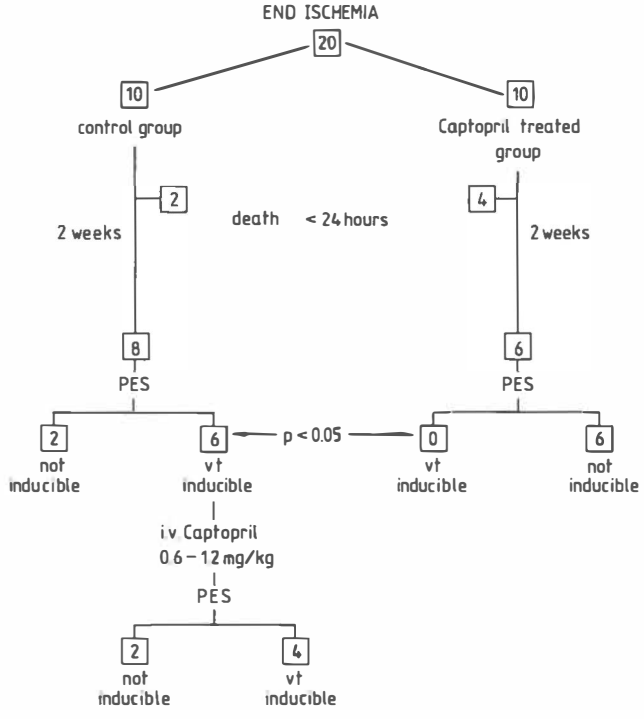

Figure 3. Flow chart of the successive phases. Programmed electrical stimulation (PES) was performed after 2 weeks. The numbers within the boxes represent numbers of animals.

Discussion

The results of this study demonstrate that captopril can beneficially influence the development of severe ventricular arrhythmias resulting from ischemia and subsequent reperfusion. However, this effect may vary, depending on the experimental or clinical situation. Our experiments in the isolated rat heart show that captopril can reduce ventricular fibrillation on reperfusion after 15 minutes of local ischemia. Concomitantly, reduction in purine overflow on reperfusion, improvement in contractility, and increase in coronary blood flow occurred. These effects were not only present when captopril was administered at the onset of the control period, but also when the drug was given only at the end of the ischemic period, although the effects were less pronounced. The ability of captopril, and also of another angiotensin converting inhibitor, ramipril, to prevent arrhythmias, to reduce cellular injury, and to ameliorate cardiac mechanical function after local ischemia and reperfusion in isolated rat hearts has been described earlier both by our group, [10,11] and by other investigators. [6] These protective effects were also present when the ani-

mals were pretreated with ramipril in vivo and the hearts were subjected to isolated perfusion. [6] This indicates that persistent changes occur, clearly localized in heart tissue and independent of pre- and afterload reduction.

Inhibition of local converting enzyme in heart tissue [7,8] appears to be responsible, since angiotensin I was no longer effective in the pretreated rats. [6] This is also shown by the fact that the in vitro effects of ramipril were beneficial only when the active, angiotensin converting enzyme-inhibiting form (ramiprilat) was given. [11] How this results in a reduction of reperfusion arrhythmias remains to be established. Previous studies have assumed an association with a demonstrated reduction of norepinephrine overflow on reperfusion. [10,11] Since these effects were abolished when indomethacin was added to the perfusate, it has been suggested that stimulation of prostacyclin by reducing bradykinin breakdown plays an important role in the underlying mechanism. [10] Other investigators have put more emphasis on the inhibition of locally generated angiotensin II, although they also found bradykinin-mediated protection against reperfusion arrhythmias. [6]

Furthermore, it might be speculated that the protective effects of captopril are at least partly due to its potential as a scavenger of free radicals, since captopril possesses a sulfhydryl group. Generation of oxygen-derived free radicals can play an important role in the pathogenesis of cardiac ischemic injury and the exacerbation by reperfusion. [18] This may explain why beneficial effects occurred even when captopril was given just before reperfusion. However, these effects were smaller compared with those of early treatment, and more than one mechanism appears to be involved, as is also demonstrated by the beneficial effects of other, non-sulfhydryl angiotensin converting enzyme inhibitors.

In contrast with the aforementioned findings, we were unable to show any effect of captopril on reperfusion arrhythmias in our closed- chest pig model. However, only an accelerated idioventricular rhythm occurred, and no ventricular fibrillation was seen. This was related to the longer duration of ischemia (60 minutes) compared with that in the experiments in the isolated rat hearts (15 minutes). The decline in vulnerability to ventricular fibrillation is due to the onset of irreversible cellular injury, which occurs between 15 and 30 minutes of ischemia. [19,20] An accelerated idioventricular rhythm is often seen during successful thrombolytic therapy and is generally not associated with significant hemodynamic compromise. [21] This arrhythmia appears unrelated to cellular damage and norepinephrine overflow. It marks the restoration of antegrade flow in a previously occluded coronary artery and is probably due to enhanced automaticity. [21]

When programmed electrical stimulation was performed after two weeks, a marked preventive effect of captopril on the inducibility of ventricular tachycardia was noted. Apparently, early intervention with captopril can reduce the

vulnerability of the injured ischemic myocardium to severe ventricular rhythm disturbances during the chronic phase. Since captopril does not substantially affect the cardiac action potential, other causative factors must be involved. An important finding in this regard is the reduction of myocardial damage during reperfusion, as demonstrated by a reduction in creatine kinase release, when captopril was given at the end of the ischemic period. This is accordance with earlier experiments. [14] The underlying mechanisms have already been discussed. [14] However, it remains to be determined whether or not salvage of ischemic myocardium results in a reduction of ventricular arrhythmias during the subacute and chronic phase of myocardial infarction. Maximal creatine kinase levels during the acute phase of myocardial infarction were lower in the animals without inducible ventricular tachycardia compared with the pigs, which were inducible, but this difference was not statistically significant. This also applies to the altered hemodynamic function, which was demonstrated in our study by a significant reduction of the pressure-rate index in the captopril-treated animals. Interestingly, acute-phase intervention with intravenous captopril also showed an inhibitory effect on the inducibility of ventricular tachycardia after two weeks in two of the six untreated animals. This is in accordance with earlier results, and it has been suggested that inhibition of angiotensin II formation and subsequent inhibition of norepinephrine release are responsible for this effect. [9] Although several factors may contribute to the protective effect of captopril on inducible ventricular tachycardia, both limitation of infarct size and suppression of norepinephrine release seem to be of major importance.

So far, no clinical studies in patients with ischemic heart disease have addressed the question of whether or not treatment with angiotensin converting enzyme inhibitors may influence the susceptibility to ventricular arrhythmias. Three studies in patients with congestive heart failure, including patients with ischemic heart disease, showing a reduction in ventricular arrhythmias when captopril or enalapril was given have been published. [3-5] It has been suggested that this may be due to an increase in serum potassium and/or a decrease in plasma catecholamine levels. [1-5] We recently completed an open, randomized, parallel study in 12 patients with moderate to severe congestive heart failure, comparing captopril with ramipril for a period of three months. Heart rhythm was monitored by 24-hour ambulatory electrocardiography before and after six and 12 weeks treatment. In neither group was a significant effect on ventricular arrhythmias noted, despite the fact that severe ventricular rhythm disturbances were present in the majority of patients. Two patients died suddenly. This lack of effect may be due to the fact that serum potassium concentration was already in the normal range in all patients before treatment (mean, 4.1 mmol/liter), without further increase thereafter. Further prospective studies are needed to determine whether inhibition of angiotensin II can reduce ven-

tricular arrhythmias in patients with end-stage congestive heart failure by mechanisms independent of its aldosterone-mediated reversal of electrolyte deficits.

From our results, we conclude that the greatest benefits of angiotensin converting enzyme inhibitors with regard to the development of ventricular arrhythmias can be achieved by early intervention during the course of ischemic heart disease. Salvage of ischemic myocardium, improved left ventricular function, beneficial effects on coronary flow, improved neurohumoral activity, and reversal of electrolyte deficits may all contribute to this effect. Since ventricular arrhythmias are an independent risk factor for sudden death in ischemic heart disease [22], the eventual aim of such treatment will be an improvement in survival. [1,2,23-25] Ongoing clinical trials with captopril and other angiotensin converting enzyme inhibitors, both in patients with acute myocardial infarction and in patients during early congestive heart failure, will hopefully give a positive answer to the question of whether or not this goal can be achieved.

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APPENDIX 7

CAPTOPRIL AS A POSSIBLE CARDIO-PROTECTIVE AGENT DURING THROMBOLYTIC THERAPY IN ACUTE MYOCARDIAL INFARCTION: DOSAGE AND CLINICAL AND NEUROHUMORAL EFFECTS

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Submitted for publication

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Summary

Captopril reduces reperfusion injury in experimental models. Its acute clinical effects were studied in patients with acute myocardial infarction who underwent thrombolytic therapy with intravenous streptokinase (1,500,000 IU in one hour). Eight patients received concomitantly 0.5-12.5 mg captopril intravenously and 11 others 3 mg captopril orally. Effects were compared with a control group (n=5). Patency rates were comparable among all groups. After intravenous captopril, all patients showed a short-lasting decrease in mean arterial pressure from 109 ± 18 to 47 ± 11 mmHg, with a maximum after 19 ± 10 min, even at low dose. After oral captopril, the mean arterial pressure decreased from 99 ± 13 to 67 ± 18 mmHg, with a maximum after 35 ± 20 min, comparable with the control group (94 ± 17 to 73 ± 13 mmHg after 32 ± 12 min). No reflex tachycardia was observed. Plasma renin activity increased significantly in the captopril treated groups. Noradrenaline levels remained unchanged after intravenous captopril and decreased significantly after oral administration (from 969 ± 98 to 515 ± 87 $pg.ml^{-1}$). The occurrence of nonsustained ventricular tachycardia during the first four hours tended to be lower in captopril treated patients as compared with the actual and a historical control group (n=23).

Thus, an appropriate dose of intravenous captopril could not be established, possibly because of a bradykinin mediated interaction with streptokinase. However, oral captopril (3 mg) could be administered without serious side effects. Patients receiving thrombolytic therapy may benefit from oral captopril by reduced angiotensin II and subsequently decreased noradrenaline release.

Introduction

It has been established that the renin-angiotensin-aldosterone system is activated following acute myocardial infarction. [1-3] This activation may be associated with serious ventricular arrhythmias, [1] the extension of myocardial damage, [2] and the development of heart failure. [3] Therefore, it was suggested that pharmacological blockade of the renin-angiotensin-system by inhibitors of the angiotensin converting enzyme such as captopril and enalapril may lead to an improvement in the prognosis of myocardial infarction. [1-3] Clinical experience has been limited so far to patients with acute myocardial infarction with left ventricular failure. [4-6] Two recent studies demonstrated a beneficial effect of captopril on symptomless ventricular dysfunction after transmural myocardial function, but treatment was not started until after one week. [7,8]

Most of the data on the effect of converting enzyme inhibitors in acute myocardial infarction have been derived from animal experiments. These indeed showed beneficial effects, [9,10] but there is still some controversy. [11,12] It has been shown in both the isolated rat heart and a closed chest pig model that captopril can reduce myocardial damage during reperfusion after myocardial ischaemia. [13,14] In the clinical situation, reperfusion can be achieved by early institution of thrombolytic therapy, leading to reduction in infarct size, improvement in myocardial function and reduction in mortality. [15] However, despite these obvious benefits, many investigators have been increasingly concerned that reperfusion itself may sometimes cause myocardial injury, which blunts the beneficial effect. [16] Agents which, when administered at the time of reperfusion, will minimize myocardial injury upon thrombolysis and prevent subsequent increase in infarct size should be investigated. [17]

For these reasons, we decided to perform a clinical study with captopril in patients with acute myocardial infarction who were treated with thrombolytic therapy. The objective of the study was to establish an appropriate dosage of captopril and to obtain initial data on clinical and neurohumoral effects in these patients.

Methods

Patient selection

We studied 24 consecutive patients of less than 71 years of age who were admitted to our hospital with acute myocardial infarction. Selection criteria included the presence of characteristic symptoms of acute myocardial infarction less than four hours after the onset of chest pain and at least 1 mm ST segment elevation in two inferior leads or 2 mm ST-segment elevation in two or more precordial leads of the 12-lead electrocardiogram. All patients showed subse-

quent increases in serum creatine kinase levels. Patients were excluded if using an angiotensin converting enzyme inhibitor or if severe renal insufficiency (serum creatinine $\geq 300\ \mu mol.l^{-1}$), severe hypertension or hypotension (systolic blood pressure ≥ 200 mmHg or ≤ 100 mmHg; diastolic blood pressure ≥ 120 mmHg or ≤ 60 mmHg), signs and symptoms of severe heart failure (Killip III and IV) or serious systemic disease were present or if contraindications to thrombolytic therapy existed. Informed consent was obtained from all patients before they entered the study.

Treatment protocol

After admission to the coronary care unit, 1,500,000 IU of streptokinase was administered by continuous intravenous infusion during one hour. Concomitantly with streptokinase, the first eight patients received captopril intravenously. The blood pressure and the heart rate were monitored during the first hours with an automatic cuff blood pressure measurement device (Dynamap) with digital print-out every 2-3 min during the first hour. It was planned to start with a dose of 2.5 mg captopril followed after 5 min by a bolus infusion of 10 mg, which was to be repeated after 30 min. If after any dose of captopril blood pressure decreased to below 100 mmHg systolic and/or 60 mmHg diastolic, the administration of captopril was discontinued. The next eleven patients were treated orally with a 3 mg capsule of captopril administered immediately prior to streptokinase infusion; this dosage was repeated after one hour if the first dose was well tolerated. All captopril-treated patients received maintenance treatment, starting with 6.25 mg of oral captopril after 4-8 hours and 25 mg three times daily thereafter. Five patients subjected to the same protocol did not receive captopril during and after thrombolytic therapy and served as controls.

Serum creatine kinase levels were determined before starting the streptokinase infusion, after 12 and 24 h and every morning during the next 3 days.

Plasma renin activity and noradrenaline levels

Venous blood was collected from all patients for the determination of plasma renin activity and noradrenaline before and 30 and 60 min after the start of captopril administration. Blood samples were collected in EDTA containing tubes and kept in melting ice until cooled centrifugation within 20 min. Plasma was stored at –70°C until assay. Established techniques were used for the assays. [18,19]

Analysis of arrhythmia recordings

The occurrence of ventricular arrhythmias was assessed by two-channel ambulatory monitoring during the first four hours after the start of captopril treat-

ment. The initial analysis was performed semiautomated (Reynolds Medical Pathfinder 3). During a second analysis only the number of episodes of accelerated idioventricular rhythm and ventricular tachycardia was determined by real time counting, whereas premature ventricular beats were disregarded. Accelerated idioventricular rhythm was defined as a repetition of 3 or more monomorphic ventricular beats with a rate of less than 100 beats per min. Ventricular tachycardia was defined as a repetition of 3 or more ventricular beats with a rate exceeding 100 beats per min. The occurrence of these arrhythmias within the intravenous and oral groups was compared with that of the actual and a historical control group.

Table I:
Clinical data on the patients who received captopril intravenously (A) or orally (B) or who served as control (C).

	A Captopril intravenously	B Captopril orally	C Controls
Number	8	11	5
Age	58(33-69)	57(36-66)	53(28-70)
Male/Female	7/1	3/8	5/0
Site of Infarction:			
Anterior/lateral	3	6	2
Inferior/posterior	5	5	3
Peak creatine kinase (U/l)	1291 ± 280	1553 ± 339	1099 ± 200
Reperfusion*:			
TIMI class 0	2	3	1
TIMI class I	2	3	1
TIMI class II	0	0	0
TIMI class III	4	5	3

*Classification according to Thrombolysis in Myocardial Infarction (TIMI) trial (20).

The historical control group consisted of 23 consecutive patients who were treated on the basis of identical entry criteria with the same intravenous streptokinase regimen but without captopril, as part of a previous streptokinase dos-response study. [21]

Coronary angiography

Coronary angiography was performed within 3 hours after streptokinase infusion to verify the site of infarction and to see whether patency of the infarct related vessel was obtained. Patency was estimated according to the classification used in the Thrombolysis in Myocardial Infarction (TIMI) Trial. [20]

Statistical analysis

Statistical evaluations were done by using the Student t test or Wilcoxon matched pairs test. Group differences with a p value of less than 0.05, double-sided, were considered significant. Results are given as mean values ± standard error of the mean.

Results

Patient characteristics are listed in Table I. The time between the onset of symptoms and the administration of streptokinase was 2.4 ± 0.2 h and 3 ± 0.6 h in the groups which received concomitant treatment with intravenous and oral captopril, respectively, and 2.9 ± 0.2 h in the control group. Reperfusion rates were comparable among the three groups (Table 1). Creatine kinase levels were not significantly different in the three groups.

Hemodynamic effects

Mean arterial pressure and heart rate are shown in Figure 1. Changes in systolic and diastolic blood pressure during the first hour after administration of captopril are given in Table 2.

Due to a very rapid and drastic decrease in blood pressure, the intravenous dose of captopril had to be reduced in each consecutive patient. The first three patients received a bolus injection of 2.5 mg, followed by a second bolus of 10 mg after 5 min in the first and 2.5 mg in the second patient. In the third one, the second bolus was replaced by a continuous infusion of 0.25 $mg.min^{-1}$. The remaining five patients were treated from the start by intravenous infusion at an initial rate of 0.25 $mg.min^{-1}$ in one patient and 0.1 $mg.min^{-1}$ in four others, the total dose varying from 0.5-3.9 mg. Despite reductions in dose levels, all patients showed a very acute reduction in blood pressure after 20 ± 4 min (Table 2). Mean arterial pressure was significantly lower than in the control

Table II:

Blood pressure (BP) changes during the first hour after administration of captopril

	Control	i.v. Captopril	oral Captopril
Baseline:			
Systolic BP	140 ± 15	150 ± 9	130 ± 7
Diastolic BP	71 ± 5	88 ± 6	88 ± 16
Maximal effect:			
Systolic BP	106 ± 10*	69 ± 6*#	97 ± 6*
Diastolic BP	56 ± 4*	33 ± 4*#	60 ± 6*
60 min after captopril:			
Systolic BP	117 ± 8	122 ± 9	121 ± 5
Diastolic BP	65 ± 8	75 ± 5	76 ± 5
Time at maximal effect(min)	33 ± 9	20 ± 4	32 ± 4

BP = blood pressure
* $p \leq 0.05$ as compared with baseline value
# $p \leq 0.05$ as compared with control group

group. However, the decrease in blood pressure was shortlasting and blood pressure was rapidly restored to normal values. The decrease in blood pressure necessitated the use of intravenous angiotensin II in two patients. Despite the severe decrease in blood pressure, the heart rate remained unchanged (Figure 1) and the patients showed no serious hypotensive symptoms, especially no dizziness or fainting.

Of the 11 patients who were treated with 3 mg captopril orally, only one developed symptomatic hypotension. All, except this patient, received a second dose of 3 mg at the end of the streptokinase infusion and without clinical problems. The blood pressure decreased significantly (Table 2) and was maximal after 32 ± 4 min. This change was not significantly different from that of the

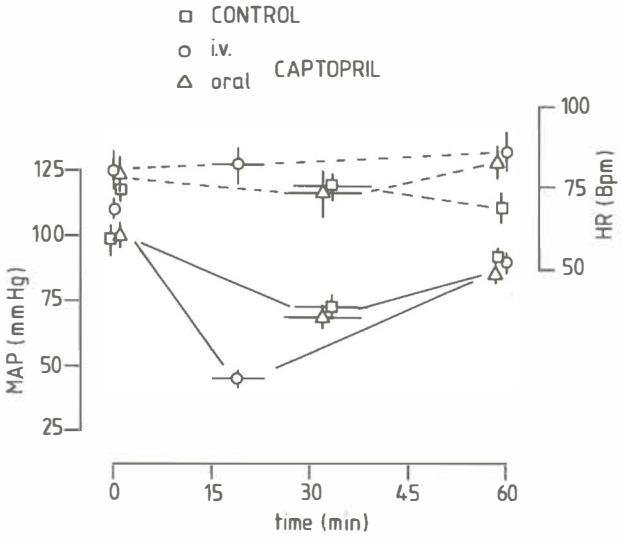

Figure 1. Changes in mean arterial pressure (MAP) and heart rate (HR) before and during one hour of streptokinase infusion in the control group and in the groups which concomitantly received captopril intravenously or orally. Symbols are mean ± SEM.

control group. At the end of the streptokinase infusion, the blood pressure was fully restored. As shown in Figure 1, the heart rate did not change significantly.

Neurohumoral changes

The plasma renin activity during infusion with streptokinase is shown in Figure 2. Both treatment groups showed a significant increase in plasma renin activity and this was most pronounced after intravenous captopril.

Changes in noradrenaline levels are shown as relative changes in Figure 3. The levels at the start of the streptokinase infusion were not significantly different among the groups (mean: 760 ± 60 pg.ml^{-1} ; control 521 ± 44 pg.ml^{-1}; captopril i.v. 666 ± 65 pg.ml^{-1}; captopril p.o. 995 ± 105 pg.ml^{-1}). At the end of the streptokinase infusion, noradrenaline levels were significantly reduced to 50% of the initial values in the oral captopril group; no significant changes occurred in the group which received intravenous captopril or in the control group (Figure 3). The two patients in the intravenously treated captopril group who received intravenous angiotensin II showed a marked increase in noradrenaline from 416 and 646 pg.ml^{-1} to a maximum of 592 and 2774 pg.ml^{-1}, respectively. In the remaining six patients, noradrenaline levels were 711 ±73 pg.ml^{-1} at the start of the thrombolytic treatment, 763 ± 91 pg.ml^{-1} after 30 min and 773 ± 87 pg.ml^{-1} after 60 min.

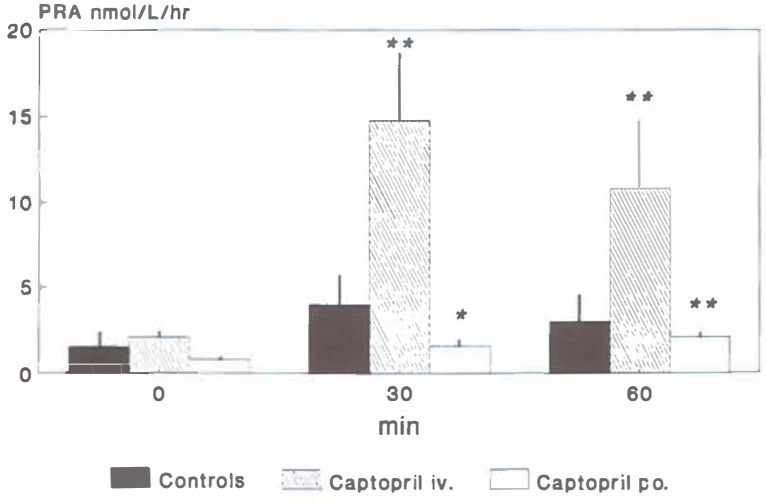

Figure 2. Changes in plasma renin activity (PRA) before (0) and after 30 and 60 min of streptokinase infusion in the control group and in the groups which concomitantly received captopril intravenously or orally. Symbols are mean ± SEM.
*$p < 0.05$; **$p < 0.01$, compared with values at t=0.

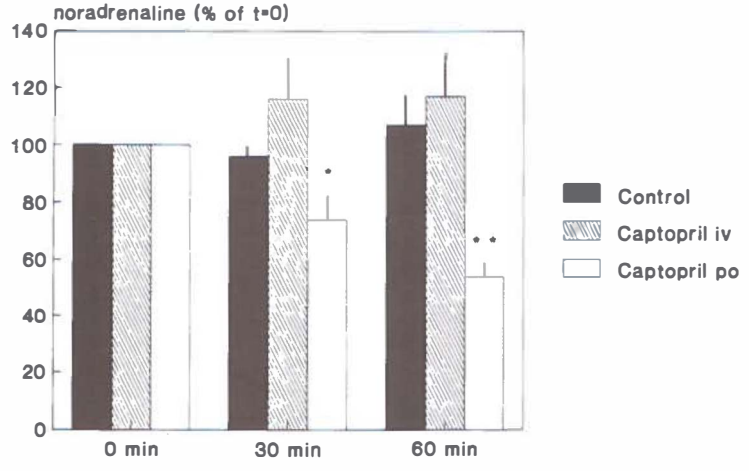

Figure 3. Relative changes in noradrenaline (NA) before (0) and after 30 and 60 min of streptokinase infusion in the control group and in the groups which concomitantly received captopril intravenously or orally. Symbols are mean ± SEM.
*$p < 0.05$; **$p < 0.01$, compared with values at t=0.

Ventricular arrhythmias

From 17 patients ambulatory ECG recordings were obtained during the first four hours after start of therapy. There were 2 recording failures in the orally treated captopril group. Results are summarized in Table 3. As compared with the patients not receiving captopril during thrombolytic therapy, a reduction in the occurrence of nonsustained ventricular tachycardia was observed in the treated group, whereas the occurrence of accelerated idioventricular rhythm did not appear to be changed by treatment.

Tabel III

Occurrence of accelerated idioventricular rhythm (AIVR) and nonsustained ventricular tachycardia (NSVT) during the first four hours after start of treatment.

	Number	NO AIVR/NSVT	AIVR	NSVT
Captopril treated patients	17	13	4*	1*
Study control group plus historical control group	28	22	3#	5#

* One patient had both AIVR and NSVT
\# Two patients had both AIVR and NSVT

Discussion

This is the first clinical study in which captopril was administered to patients with acute myocardial infarction during thrombolytic therapy. The primary objective was to evaluate the feasibility of this combined intervention and to obtain initial data on the potentially beneficial effects in these patients.

It is clear from our results that no appropriate dosage of intravenous captopril was found in the dose range which was used. Despite the fact that the dosage was decreased in all subsequent patients, an acute and dramatic decrease in blood pressure was observed in all eight patients, even at an infusion rate of 0.1 $mg.ml^{-1}$ captopril during 5 minutes. Our initial dosage schedule was based on a previous study in patients with severe heart failure, where a dose range of 0.5-5.0 mg was advised to assess the response in patients on intravenous treat-

ment. [22] After oral treatment with 3 mg captopril, the hypotensive effect was much less pronounced and comparable with changes in blood pressure in the control group.Hypotension after institution of the thrombolytic therapy occurred significantly later than in the intravenously treated group.

There are several explanations for the hypotension observed after intravenous captopril. The renin-angiotensin-aldosterone system is activated following acute myocardial infarction. [1-3] This is further enhanced by thrombolysis, as is demonstrated in our study by the marked increase in the plasma renin activity during this treatment. Since there is a significant relationship between the activity of the renin-angiotensin-system and the magnitude of the acute decrease in blood pressure after the first dose of captopril, [23] hypotension will follow. The lesser hypotensive effect in the patients treated with oral captopril can be explained by less activation of the renin-angiotensin system at baseline, as demonstrated by the lower PRA levels at the start of this treatment. However, the changes in plasma renin activity may also be secondary to the decrease in blood pressure. This decrease in blood pressure was much more pronounced than that observed in animal experiment [14] and in patients with severe heart failure, who also show a marked activation of the renin-angiotensin system. [22]

Therefore, other mechanisms may be involved. One of them could be an interaction between streptokinase and captopril through potentiation of bradykinin. It is known that streptokinase promotes conversion of plasminogen into plasmin, which in its turn generates bradykinin from high molecular weight kininogen by activating the conversion of prekallikrein to kallikrein. [24] This increase in bradykinin may be the reason why hypotension is frequently seen after thrombolysis by streptokinase alone, as is also shown in our control group. [25] On the other hand, captopril potentiates bradykinin by inhibition of the angiotensin enzyme. [26] Therefore, when streptokinase and captopril are administered simultaneously, potentation of the effect of bradykinin will occur, with a sharp and rapid decrease in blood pressure. When the inhibition of the angiotensin converting enzyme occurs more slowly, as is the case after oral captopril, adaptation to the effect of streptokinase on bradykinin may occur, which could explain the less pronounced hypotensive response after oral treatment. However, more studies are needed to clarify the underlying mechanism of this possible interaction between captopril and streptokinase or other thrombolytic agents.

Interestingly, despite the decrease in blood pressure after intravenous captopril, there was no increase in heart rate. This is in agreement with the observation that no significant increase of noradrenaline levels occurred. Noradrenaline levels even decreased significantly after oral captopril. Of course, in view of the number of patients studied, care should be taken in extrapolating these figures. Nevertheless, if true, the finding of decreased noradrenaline le-

vels may be of clinical significance, since it has been suggested that the magnitude of sympathicoadrenal activation early in the course of clinical acute myocardial infarction is related to the extent of myocardial damage and late mortality. [27] Increased baseline levels of noradrenaline, which have been demonstrated after myocardial infarction, [27-29] were also found in our study before treatment was started. Noradrenaline levels may further increase during thrombolysis and subsequent reperfusion, as was shown in our animal experiments. [13,14] This effect can be partly mediated by activation of the renin-angiotensin system, since angiotensin II can stimulate and potentiate sympathetic nervous system activity. [30] Conversely, inhibition of angiotensin II by captopril can lead to lower noradrenaline levels, [31] as was also shown in our study. Eventually this reduction may beneficially influence the clinical outcome of the acute myocardial infarction, as shown earlier with beta-adrenergic blocker treatment. [15]

The reduction in angiotensin II can also itself be beneficial, since stimulation of angiotensin II can induce coronary vasoconstriction, increase myocardial oxygen consumption and extend myocardial necrosis, which is deleterious in the case of acute myocardial infarction. [4,32] Although we did not measure angiotensin II levels in the plasma, suppression is strongly suggested by the (reciprocal) increase in plasma renin activity. Furthermore, an oral dose of 3 mg captopril has been shown to lead to a significant inhibition of angiotensin II in acute left ventricular failure complicating myocardial infarction. [6]

Captopril may also exert its beneficial effect, at least theoretically, by scavenging free radicals. Toxic free radicals are generated at the time of and shortly after reperfusion. The termination of free radicals may play an important role in the development of reperfusion injury. [17] Animal experiments have shown that captopril can remove these free radicals due to the presence of a sulfhydryl group. [33] This awaits further investigation.

An oral dose of 3 mg captopril seems to be an appropriate dosage with potentially beneficial effects in patients with acute myocardial infarction undergoing thrombolysis. No significant differences in maximal creatine kinase levels were seen, despite the marked hypotension after intravenous captopril. Accelerated idioventricular rhythm, which is generally benign, [34] occurred in the same incidence as in the historic control group, whereas nonsustained ventricular tachycardia was seen in only one patient as compared with five patients in the historical control group. However, due to the limited number of patients and the design of the study, no definite conclusions can be made with regard to clinical effects on myocardial damage and reperfusion arrhythmias. A large scale, placebo-controlled trial in patients with acute myocardial infarction treated with thrombolytic therapy is therefore necessary to determine whether treatment with captopril will enhance salvage of ischemic myocardium and eventually improve myocardial function and prolong survival.

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APPENDIX 8

Letter to the Editors

POTENTIATION OF ISOSORBIDE DINITRATE-INDUCED CORONARY DILATATION BY CAPTOPRIL

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J Cardiovasc Pharmacol 1987;9:254-255.

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It has been suggested that interactions with tissue sulfhydryl groups in vascular smooth muscle are involved in the mechanism of action of organic nitrates. This interaction activates guanylate cyclase, resulting in increased intracellular concentrations of cGMP, which in turn induces vasodilatation. [1] Therefore, the availability of sulfhydryl groups and/or their redox states may be determinants of both responsiveness to organic nitrates and tolerance for these compounds. Both under experimental conditions [2,3] and in the clinical situation, [4] it has been demonstrated that sulfhydryl group "donors", such as N-acetylcysteine, potentiate the hemodynamic responses to nitrates. Furthermore, these compounds are capable of reversing nitrate tolerance. [2]

The angiotensin-converting enzyme inhibitor captopril is another sulfhydryl-containing compound. It is widely used in the treatment of patients with hypertension and congestive heart failure. In such patients, ischemic heart disease is not uncommon, and thus organic nitrates are often used in patients receiving captopril. However, no previously published report has evaluated the influence of captopril on the coronary vascular response to organic nitrates. Therefore, the present study was performed to explore the effect of captopril on the vasodilatory response to isosorbide dinitrate in an isolated rat heart model. This was compared with the effect of cysteine, a sulfhydryl-containing compound without an effect on the angiotensin-converting enzyme, and with the effect of ramiprilat, an angiotensin-converting enzyme inhibitor without a sulfhydryl moiety.

The isolated hearts from male Wistar rats (275-325 g) were perfused as described by Langendorff at a constant pressure of 60 mm Hg. After equilibra-

tion, the hearts were subjected to three different treatment periods of 10 minutes, separated by washout periods of 10 minutes.

The hearts were divided at random into four groups of six each. Group A received, during each treatment period, 8 μg/ml captopril or 40 μg/ml isosorbide dinitrate or both drugs combined. Group B received 4.4 μg/ml cysteine or 40 μg/ml isosorbide dinitrate or both. Group C received 1.5 μg/ml ramiprilat or 40 μg/ml isosorbide dinitrate or both. The sequence of treatment was randomized and each sequence was applied only once. Six hearts served as controls.

Coronary flow was measured by a microprocessor, and a constant perfusion pressure was maintained by adjusting the perfusion pump. Furthermore, flow

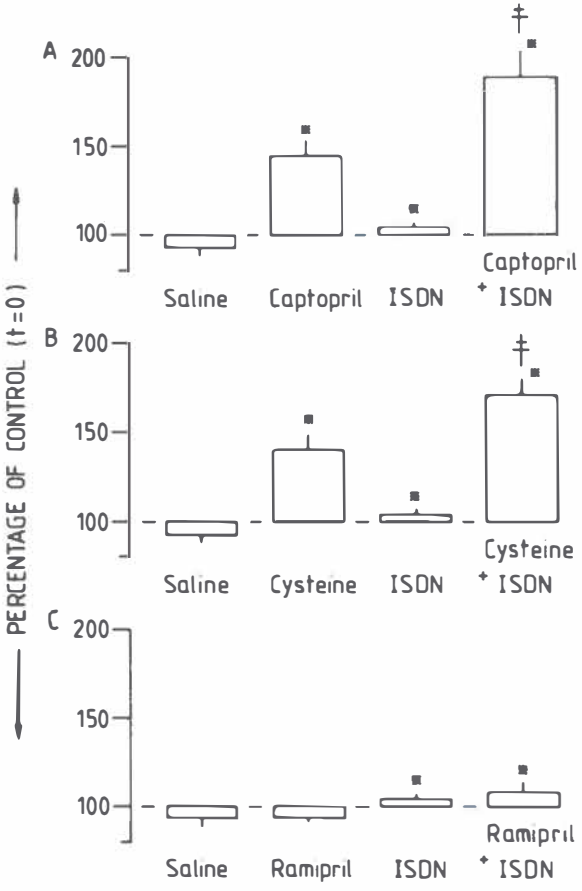

Figure 1. Changes in coronary flow at the end of the treatment period (t = 10) are shown as percentages of control values (t = 0). Asterisk indicates significant increase when compared with the saline group. Double dagger indicates significant increase when compared with the sum of the effect of both compounds alone. ISDN = isosobide dinitrate. Ramipril is active ramiprilat.

was checked by collecting 1-minute samples every 5 minutes. Left ventricular pressure was measured by means of a catheter inserted into the left ventricle and connected to a pressure transducer. A bipolar cardiac electrogram was obtained by means of two silver electrodes - one attached to the metal inflow cannula and the other to the ventricular apex. Heart rate was continuously registered. Differences were tested using Student's paired and unpaired t tests and considered to be significant at p values of less than 0.05. After equilibration, the baseline values for measured parameters were comparable for all groups. At the end of the treatment period there were no significant differences in heart rate and left ventricular pressure values among the groups (data not shown). Figure 1 shows the effects of different treatments on coronary flow. Captopril, cysteine, and isosorbide dinitrate alone produced a significant increase. [5]Ramiprilat alone had no significant effect. Both the combination of captopril with isosorbide dinitrate and that of cysteine with isosorbide dinitrate showed a significant potentiation of the effect on coronary flow, which was not observed for the combination of ramiprilat and isosorbide dinitrate.

These results indicate that captopril can potentiate the effect of isosorbide dinitrate. This effect is not due to the angiotensin-converting enzyme-inhibiting properties of captopril, since it is not evoked by ramiprilat in equipotent concentrations. Probably, the presence of a sulfhydryl group in captopril is responsible for this interaction, since a comparable effect is observed for equimolar concentrations of cysteine. To the best of our knowledge, this is the first report on such an interaction. In view of the clinical use of captopril and organic nitrates, this observation may have important consequences for the treatment of patients with ischemic heart disease.

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APPENDIX 9

ANTIANGINAL EFFECTS OF CAPTOPRIL DURING CHRONIC NITRATE TREATMENT

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Summary

Several clinical studies have shown that the sulfhydryl containing compound N-acetylcysteine enhances the vasodilating effects of nitrate therapy. Experimental work has shown similar effects for captopril, a sulfhydryl-containing angiotensin converting enzyme inhibitor. Therefore, the effect of captopril was studied on graded exercise testing in patients with stable, exercise-induced angina pectoris during long term nitrate treatment. Ten patients were treated for three weeks with slow release isosorbide-dinitrate (20 mg four times daily). At day 7, a baseline exercise test was obtained. Subsequently, patients with chest pain and at least 1-mm ST-depression on the ECG during exercise were included. They received on days 14 and 21 either captopril (25 mg) or placebo one hour before exercise testing in a randomized, double-blind, cross-over design. Captopril significantly improved the combined score of maximal ST-depression (0.18 ± 0.03 versus 0.23 ± 0.05 mm), maximal workload (137 ± 5 versus 125 ± 8 Watt) and time to angina (8.5 ± 0.5 versus 7.2 ±0.7 min), compared to placebo. No differences in pressure-rate index at rest and during exercise were observed.

In conclusion, captopril may improve exercise performance in patients with stable angina pectoris during chronic nitrate treatment. Sulfhydryl-dependent potentiation of nitrate therapy and reversal of nitrate tolerance may be underlying mechanisms.

Introduction

Organic nitrates are widely employed in the management of patients with angina pectoris and congestive heart failure (1,2). Their therapeutic effects appear to be secondary to their vasodilating effects on the systemic and coronary vasculature. Although the mechanism of action is not completely understood, it is assumed that nitrates cause vasodilation by interacting with sulfhydryl groups in vascular smooth muscle eventually leading to increased intracellular concentrations of cyclic guanosine monophosphate (3,4). Prolonged or frequent administration of nitrates can lead to decreased availability of sulfhydrylgroups which may result in vascular tolerance and loss of therapeutic efficacy (5,6). It has been shown repeatedly, that administration of the sulfhydryl containing compound N-acetylcysteine enhances nitrate vascular effects, both in the tolerant and non-tolerant state (7-12).

The oral angiotensin converting enzyme inhibitor captopril, which has become very popular in the management of hypertension and congestive heart failure, is another sulfhydryl-containing compound (13). Thus, captopril may enhance the effects of nitrates, both after initial and maintenance treatment. This was investigated in experiments in the isolated rat heart which demonstrated that captopril can potentiate the coronary vascular response to isosorbide dinitrate (14). In contrast, ramiprilat, an angiotensin converting enzyme inhibitor without a sulfhydryl moiety, had no significant effect (14)

The present study was undertaken to investigate whether these effects of captopril are also relevant in the clinical situation. For this purpose, a single dose of captopril was given to patients with stable angina pectoris who already received a maintenance dose of isosorbide dinitrate.

Methods

Ten patients, nine male, one female, ranging in age from 48 to 65 years (average, 55.7) with chronic, stable exertional angina pectoris of at least one month duration were enrolled in this investigation. Informed consent was obtained from the patients, and the research protocol was approved by the ethics committee of the Wilhelmina Hospital at Assen. All patients had coronary artery disease as documented by previous myocardial infarction or coronary arteriographic abnormalities (at least 60% obstruction in one or more major coronary arteries). All patients had a positive exercise tolerance test as defined by the development of chest pain during exercise and ischemic ST-segment depression ($\geqslant$ 1-mm horizontal or downsloping ST-segment depression persisting for at least 80 msec after the J point on the electrocardiogram).

Exercise testing was performed on a bicycle ergometer. The workload started at 50 Watt and was increased every 3 minutes by 30 Watt, until discontinuation. Patients were asked to indicate the moment of onset of angina and the reason for discontinuation. A 12-lead standard electrocardiogram was recorded immediately prior to exercise, at one minute intervals throughout exercise, at maximal exercise, and after termination of the test. The absolute ST segment deviation at maximal exercise was measured. Blood pressure and heart rate were recorded at the same time intervals. The pressure-rate index was determined by multiplying systolic blood pressure and heart rate.

Patients were excluded if they had suffered a myocardial infarction within three months, unstable angina pectoris, evidence of congestive heart failure, severe hypertension or hypotension (systolic blood pressure $\geq$ 180 mmHg or $\leq$ 100 mmHg; diastolic blood pressure $\geq$ 100 mmHg or $\leq$ 80 mmHg), or if they were taking calcium channel blockers, diuretics, psychotropic drugs, vasodilating drugs, alpha-adrenoceptor antagonists, or nitrates other than sublingual nitroglycerin. All patients except one received the beta-blocker metoprolol (100 - 200 mg daily) throughout the study.

If patients had satisfied these requirements, they received sustained-release isosorbide dinitrate 20 mg four times daily (at 7 a.m., 12 a.m., 5 p.m., and 11 p.m.), which was continued during the course of the study. One week later, exercise testing was repeated. Only patients with a second positive exercise test were enrolled in the study. Following one and two weeks, at 2 p.m., i.e. two hours after the noon dose of isosorbide dinitrate, either a single dose of 25 mg captopril or placebo was given in a randomized, double blind, cross-over design under medical supervision. Blood pressure and heart rate were measured every fifteen minutes. One hour later, at 3 p.m., exercise testing was carried out. When finished, the patient was observed for another 30 minutes.

To investigate the effects on the renin-angiotensin system, venous blood was drawn immediately before exercise for determination of plasma renin activity. This was measured by radioimmunoassay (15).

Statistical analysis was performed by using the Student t-test for paired observations (hemodynamics) and the Wilcoxon matched pairs rank test (plasma renin activity and combined analysis of parameters of exercise testing). Differences with a p value of less than 0.05, double-sided, were considered significant. Results are given as mean value $\pm$ standard error of the mean.

Results

Before enrollment in the study, all patients demonstrated chest pain and ischemic ST-segment depression of at least 1 mm during exercise testing. Total exercise time was 10.4 $\pm$0.7 minutes, time to angina pectoris 8.3 $\pm$ 0.6 minu-

tes, maximal ST-depression 0.23 ± 0.05 mm, and maximal workload 137 ± 7 Watt.

One hour after administration of not only captopril, but also placebo, there was a significant decrease in mean arterial blood pressure, from 93 ± 3 to 86 ± 3 mmHg and from 91 ± 4 to 86 ± 4 mmHg, respectively. Heart rate did not decrease significantly, from 59 ± 3 to 57 ± 4 beats per minute and from 59 ± 3 to 57 ± 3 beats per minute, respectively. Prior to exercise testing, no significant differences were present in pressure rate-index between placebo and captopril.

Individual results during exercise testing are depicted in Table 1. Following captopril treatment, total exercise time increased from 9.7 ± 0.7 to 10.4 ± 0.5 minutes, maximal ST-depression decreased from 0.23 ± 0.05 to 0.18 ± 0.03 mm, and maximal workload increased from 125 ± 8 to 137 ± 5 Watt, compared to placebo. Time to angina pectoris increased from 7.2 ± 0.7 to 8.5 ± 0.5 minutes in 8 patients. One patient developed chest pain after placebo only. Two patients did not experience any chest pain after captopril or placebo and terminated exercise testing due to tiredness. Combined analysis of the effects on maximal ST-depression, maximal workload, and time to angina pectoris, on a three point rating scale showed a significant improvement in exercise performance after captopril administration. Maximal pressure-rate index was 16,877 ± 1,092 mmHg.bpm after captopril and 16,485 ± 1,368 mmHg.bpm af-

Tabel 1.
Individual data on exercise testing following administration of placebo (P) and captopril (C).

	total exercise time (min)		time to angina (min)		maximal ST-depression (mm)		maximal workload (W)	
	P	C	P	C	P	C	P	C
nr								
1	11	10	9	9	0.1	0.1	140	140
2	13	12	10	8	0.25	0.2	170	140
3	9	9.5	6	7	0.3	0.3	110	140
4	12	14	9	11	0.1	0.1	140	170
5	8	10	6	8	0.2	0.2	110	140
6	12	11.5	–*	–*	0.5	0.4	140	140
7	10.5	9.5	–*	–*	0.1	0.1	140	140
8	9	10	7	–*	0.5	0.2	110	140
9	6	8.5	5.5	8	0.1	0.1	80	110
10	7	9	5	7	0.1	0.1	110	110

* no angina pectoris during exercise testing

ter placebo. At no stage of the exercise testing significant differences were present.

Prior to exercise testing, plasma renin activity increased significantly following captopril treatment, compared to placebo (2.1 ± 0.7 versus 0.6 ± 0.1 nmol A1/l/hr, respectively).

Discussion

This study suggests that captopril can improve exercise performance in patients with chronic, exercise-induced angina pectoris, who are on a maintenance treatment of nitrates. The hypothesis behind this study was that captopril may enhance the antianginal and circulatory effects of nitrate therapy due to the presence of a sulfhydryl group in its molecule.

Attenuation of the therapeutic effects of prolonged or frequent administration of nitrates, either intravenously, orally, or transdermally, has increasingly been recognized as a clinically relevant problem (5,6). Nitrates exert their effects primarily by activating guanylate cyclase in the smooth muscle vascular cell (3,4). This activation is mediated by S-nitrosothiol derivatives, which are generated by initial reduction of organic nitrate to inorganic nitrite and subsequently, by reduction to nitric oxide. These two steps are dependent on the availability of sulfhydryl donors (4). Both experimental and clinical data have suggested that depletion of sulfhydryl groups in vascular smooth muscle is largely responsible for the occurrence of tolerance following continuous nitrate therapy (5,6). Studies with N-acetylcysteine have demonstrated that exogenous administration of sulfhydryl groups may reverse the loss of hemodynamic efficacy (8,10,11). In our study we gave on purpose a dosage scheme of isosorbide dinitrate (20 mg four times daily) that has been shown to induce loss of efficacy, especially during the second half of the dosing interval (16,17). This was further enhanced by the use of a sustained-release form, because tolerance is particularly likely to occur with constant nitrate levels (5). Reversal of nitrate tolerance by sulfhydryl repletion may explain the antianginal effects of captopril.

By the same mechanism, sulfhydryl-containing compounds, such as N-acetyl-cysteine, may potentiate the hemodynamic effects of nitrates when started concomitantly (7,9,12). This was also shown for captopril which potentiated the vasodilating effects of isosorbide dinitrate on the coronary vasculature in an isolated rat heart model (14). Comparable effects were observed for cysteine, but not for ramiprilat, another angiotensin converting enzyme inhibitor without a thiol group (14). Thus, captopril may not only restore the hemodynamic effects of nitrates, but even potentiate them.

Apart from this interaction with isosorbide dinitrate, sulfhydryl-containing compounds, such as captopril, may have vasodilating effects of their own, as

was also shown in the isolated rat heart (18). It was postulated that this effect is due to interaction of the sulfhydryl group with the endothelium- derived relaxation factor, which has been shown to be identical to nitric oxide (19). The production of endothelium-derived relaxing factor is stimulated by bradykinin (20), which in its turn is potentiated by angiotensin-converting enzyme inhibition (13). This mechanism of action has not yet been investigated clinically.

Angiotensin converting enzyme inhibitors in general may also exert antiischemic effects by reduction of the pressure-rate index and therefore of myocardial oxygen demand. This has been demonstrated both after captopril and enalapril, but results have been conflicting (21). Prior to exercise testing, we found a small, but significant reduction of blood pressure following administration of captopril, with a concomitant, reactive increase of plasma renin activity. This hypotensive effect also occurred following placebo and at no stage during exercise testing were significant differences in pressure-rate index found compared to captopril. Therefore, the effects of captopril on exercise performance appear not related to its systemic vasodilating properties.

The limitations of this study are the small number of patients and the single dose regimen. Nevertheless, a significant improvement in exercise performance was observed. More data are necessary to confirm these preliminary results. Comparative studies of a longer duration with other angiotensin converting enzyme inhibitors without a sulfhydryl group should be performed in order to clarify the importance of the thiol moiety. If this interaction between captopril and nitrates can be confirmed, it may have important therapeutic implications in patients with ischemic heart disease.

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Samenvatting en conclusies

Dit proefschrift beschrijft de effecten van het geneesmiddel captopril op het hart. Captopril werd in de jaren zeventig ontwikkeld tot de eerste angiotensine converting enzym remmer voor oraal gebruik. De basis hiervoor vormde de ontdekking dat een mengsel van peptiden uit het gif van de Zuid-Amerikaanse slang Bothrops Jararaca naast het vermogen bradykinine te inactiveren, ook in staat was de vorming van angiotensine II te remmen. Het angiotensine converting enzym (ACE), dat verantwoordelijk is voor de omzetting van angiotensine I in angiotensine II, bleek dan ook identiek aan het kininase II, dat verantwoordelijk is voor de afbraak van bradykinine. Identificatie van de aminozuur volgorde van deze peptiden leidde tot de synthese van het nonapeptide teprotide, dat echter alleen intraveneus gegeven kon worden, en tenslotte van captopril.

Remming van het ACE betekent dus enerzijds afname van het angiotensine II, anderzijds toename van het bradykinine. Angiotensine II heeft meerdere werkingen. Het werkt vaatvernauwend en bevordert de afgifte van aldosteron in de bijnieren. Tevens interfereert angiotensine II met de activiteit van het sympathische zenuwstelsel en oefent het een aantal effecten uit op het centrale zenuwstelsel. De bijdrage hiervan aan het werkingsmechanisme van captopril is grotendeels onopgehelderd. Dit geldt ook voor de remming van de afbraak van bradykinine. Bradykinine is een vaatverwijdende stof, maar de rol bij de regulatie van de bloedsomloop is onduidelijk.

Op grond van de eigenschappen van angiotensine II was het te verwachten dat remming door middel van captopril en andere ACE remmers zou leiden tot vaatverwijding. Dit effect zou nog toenemen door de potentiëring van bradykinine. Captopril bleek dan ook een bijzonder effectief middel bij de behandeling van hoge bloeddruk. Aanvankelijk werden echter ernstige bijwerkingen waargenomen, zoals huidafwijkingen, smaakstoornissen, afname van het aantal witte bloedcellen, en gestoorde nierfunctie. Een aantal van deze bijwerkingen hield verband met de aanwezigheid van een sulfhydryl groep in het molecuul van captopril. Dit leidde ertoe dat captopril slechts gegeven werd aan patienten met onbehandelbare hoge bloeddruk. Nieuwe orale angiotensine converting enzym remmers werden ontwikkeld die deze sulfhydryl groep niet bezaten, zoals enalapril, lisinopril, en ramipril. Uit later onderzoek met captopril bleek echter dat aanvankelijk veel te hoge doseringen captopril gegeven waren en dat het mogelijk was bepaalde risicogroepen te identificeren. Lagere doseringen bij patienten zonder risico resulteerden niet in verlies van werkzaamheid, maar wel in een veel acceptabeler bijwerkingen profiel.

Tegelijkertijd met de ontwikkeling van captopril en andere angiotensine converting enzym remmers deed de behandeling van patienten met hartfalen

door middel van vaatverwijders zijn intrede. Door een verlaging van zowel de voor- als de na-belasting van het hart bleek het mogelijk de haemodynamiek èn de symptomatologie te verbeteren. Het aangrijpingspunt van deze middelen was dus niet het hart zelf, maar de perifere bloedsomloop. Als krachtige vaatverwijder, met effecten op zowel het veneuze als arteriële systeem, bleek captopril bij deze indicatie bijzonder effectief. Deze effectiviteit ging echter wel gepaard met een soms ernstige daling van de bloeddruk en in sommige gevallen met gestoorde nierfunctie. Dit was te verwachten aangezien patienten met hartfalen compensatoir vaak een gestimuleerd renine-angiotensine-aldosteron systeem hebben. Over de effecten van de nieuwere angiotensine converting enzym remmers was weinig bekend, maar gezien de langere werkingsduur waren problemen zeker te verwachten.

Om dit verder te onderzoeken werd een open, vergelijkend onderzoek met een duur van drie maanden tussen captopril en de langwerkende angiotensine converting enzym remmer ramipril verricht. Hierbij lag de nadruk op de haemodynamische effecten en op de veiligheid op korte èn lange termijn. De resultaten zijn vermeld in de Appendices 1 en 2.

Ondanks soms uitgesproken hypotensie in de aanvangsfase, werden beide geneesmiddelen goed verdragen. Van belang hierbij was wel dat de dosis werd aangepast aan de klachten van de patient, ophoging van de dosering slechts gefaseerd plaatsvond, en niet gestreefd werd naar maximale remming van het angiotensine II gedurende 24 uur. In tegenstelling tot resultaten uit de literatuur, werd geen afname in incidentie en ernst van ventriculaire ritmestoornissen waargenomen. Mogelijk dat dit samenhing met de normale concentratie van het serum kalium bij aanvang van de studie. Er werd een persisterend effect op de haemodynamiek gemeten, dat correleerde met veranderingen in het plasma renine-angiotensine-aldosteron systeem. Er bestond wel een discrepantie tussen de haemodynamische veranderingen en de klinische verbetering op lange termijn. Dit zou mogelijk verklaard kunnen worden door additionele, plaatselijke effecten. Het renine-angiotensine-aldosteron systeem is namelijk niet alleen aanwezig in het bloed, maar ook in de vaatwand en in wisselende mate in andere weefsels en organen. Terwijl in de acute fase de remmende effecten van captopril op het plasma renine-angiotensine-systeem overheersen, treedt er in de chronische fase een verschuiving op naar de effecten in de vaatwand en de organen zelf.

Een van die organen is het hart. Aangenomen werd dat captopril geen directe invloed op het hart zelf uitoefende, maar alleen indirect door beinvloeding van de perifere bloedsomloop. Toch was reeds lang bekend dat er in het hart een renine-angiotensine-aldosteron systeem aanwezig was. Er waren ook andere redenen op grond waarvan een direct effect van captopril verondersteld kon worden. Potentiëring van bradykinine en interferentie met het sympathische zenuwstelsel zouden een rol kunnen spelen. Het probleem om een

direct effect van captopril in de klinische situatie aan te tonen was echter dat de effecten op voor- en nabelasting zo uitgesproken zijn, dat dit gemaskeerd kan worden.

Om dit uit te sluiten onderzochten wij het effect van captopril in het geisoleerde rattehart, geperfundeerd volgens Langendorff. Om eventuele werkingsmechanismen te activeren, werden ischaemie en reperfusie bewerkstelligd door tijdelijke onderbinding van de linker coronair arterie. Captopril en andere angiotensine converting enzym remmers werden via de perfusievloeistof toegediend en veranderingen in contractiliteit, coronaire doorbloeding, en ritmestoornissen vastgelegd. In de uitstroomvloeistof werden veranderingen in noradrenaline en purines, als maat voor de weefselbeschadiging, gemeten. De belangrijkste resultaten worden beschreven in de Appendices 3, 4 en 6.

Captopril bleek in staat om reperfusie-aritmieën effectief te doen afnemen met verbetering van de contractiliteit en afname van de weefselbeschadiging. Deze effecten waren concentratie-afhankelijk en aanwezig in een lage, therapeutische concentratie. Bij een zeer hoge concentratie waren effecten op de doorbloeding reeds waarneembaar tijdens ischaemie. De effecten van captopril gingen gepaard met een verdwijnen van de noradrenaline afgifte op het moment van reperfusie (Appendix 3). Overeenkomstige effecten werden ook gezien na toediening van ramipril, maar alleen na de actieve vorm, ramiprilaat, die in staat is het ACE te remmen. Wanneer tegelijkertijd indomethacine werd toegediend verdwenen de effecten van captopril (Appendix 4). Deze resultaten suggereren dat de gunstige effecten van captopril een gevolg zijn van beinvloeding van het prostaglandine metabolisme door potentiëring van intracardiaal aanwezig bradykinin middels remming van het lokale ACE. Hoewel remming van plaatselijk gevormd angiotensine II eveneens een onderliggend mechanisme zou kunnen zijn, kon dit niet aangetoond worden. Aangezien een gedeelte van de gunstige effecten ook aanwezig waren wanneer captopril vlak voor reperfusie werd toegediend, werd verondersteld dat binding van vrije radicalen via de sulfhydrylgroep van captopril bijdragend zou kunnen zijn (Appendix 6). Dit werd door andere onderzoekers bevestigd.

De volgende vraag was of deze effecten nu ook relevant waren in vivo. Om dit te beantwoorden, werd een nieuw model ontwikkeld in het varken. Hierbij werd tijdens narcose een dilatatiecatheter opgeschoven in de ramus descendens van de linker coronair arterie. Ischaemie en reperfusie werden opgewekt door opblazen en leeg laten lopen van de ballon. Net als in het geisoleerde rattehart werden effecten op de haemodynamiek, op het vrijkomen van noradrenaline en purine, en op de weefselbeschadiging gemeten. Hierbij werd het effect van captopril in verschillende concentraties, continu of vlak voor reperfusie bestudeerd in vergelijking met een controle groep. Vervolgens werd na twee weken in de overlevende varkens gekeken naar de opwekbaarheid van

ventriculaire ritmestoornissen door middel van geprogrammeerde electrische stimulatie. De resultaten staan vermeld in de Appendices 5 en 6.

Bij continue toediening werd opnieuw een concentratie-afhankelijk beschermend effect van captopril op de myocardbeschadiging gevonden. Dit kwam tot uiting in een significante afname van het vrijkomen van het creatine phosphokinase en de purines tijdens reperfusie (Appendix 5). Ook de uitstroom van noradrenaline was significant afgenomen. In tegenstelling tot het geisoleerde rattehart trad noch in de controlegroep, noch in de met captopril behandelde groepen ventrikelfibrilleren op na reperfusie. Wel werd in beide groepen een versneld idioventriculair ritme vastgesteld als uiting van reperfusie. Ook bij toediening van een enkelvoudige bolusinjectie vlak voor reperfusie werd een gunstig effect op de weefselbeschadiging gezien zonder invloed op deze, op zich onschuldige, ritmestoornis (Appendix 6). Wel aanwezig was een significant remmend effect van deze eenmalige toediening captopril op de opwekbaarheid van ventriculaire ritmestoornissen na twee weken. Ook intraveneuze toediening van captopril vòòr electrische stimulatie in de dieren die niet behandeld waren liet een dergelijk effect zien.

Deze resultaten maken duidelijk dat de cardioprotectieve effecten van captopril klinisch relevant kunnen zijn. Om dit te onderzoeken werd een onderzoek uitgevoerd bij patienten met een acuut myocardinfarct die thrombolyse met streptokinase ondergingen. Thrombolytische behandeling heeft tot doel de afsluiting van de kransslagader op te heffen, voordat het ischemische hartspierweefsel geheel necrotisch is geworden (bij de mens na 4-6 uur). Hierbij is dus sprake van ischaemie en reperfusie. Hoewel vroegtijdige reperfusie de grootte van het myocardinfarct, de afname van de linker-ventrikelfunctie, en de sterfte kan beperken, kan reperfusie ook nadelige effecten met zich meebrengen. Ter beperking van deze reperfusieschade kan gelijktijdige toediening van andere middelen zinvol zijn. Gelet op de bovengenoemde resultaten uit de dierstudies, kozen wij hierbij voor captopril.

De resultaten zijn vermeld in Appendix 7. Het doel van het onderzoek was primair om een veilige dosis te vinden. Tevens werd gekeken naar de invloed op de noradrenaline afgifte en het optreden van ventriculaire ritmestoornissen, om te zien of de eerdere resultaten opnieuw aangetoond konden worden. Opvallend was de uitgesproken bloeddrukdaling die optrad wanneer captopril in lage doses intraveneus en gelijktijdig met streptokinase gegeven werd. Mogelijk speelt een interactie met het fibrinolytische systeem hierbij een rol. Na orale toediening werd geen verschil in bloeddrukdaling ten opzichte van de controle groep gezien. Er was een significante afname van de noradrenaline spiegels. Overeenkomstig met de dierexperimenten werd frequent een versneld idioventriculair ritme gezien in alle groepen terwijl wèl een afname van het optreden van non-sustained ventriculaire tachycardie in de met captopril behandelde groepen werd gevonden. Hoewel preliminair, suggereren deze re-

sultaten dat captopril therapeutische mogelijkheden biedt bij het acute myocardinfarct, met name wanneer thrombolyse toegepast wordt.

Tijdens onderzoek in het geisoleerde rattehart werd onze aandacht gevestigd op verschillen tussen captopril en andere ACE remmers ten aanzien van de effecten op de coronaire doorstroming. De aanwezigheid van de sulfhydryl groep lijkt hiervoor verantwoordelijk, aangezien sulfhydryl groepen een essentiele rol spelen bij de vaatverwijding. Dit geldt zowel voor het effect van endogene vaatverwijdende stoffen als voor de haemodynamische effecten van exogeen toegediende nitraten. Als bewijs hiervoor werd een studie uitgevoerd, die is verwoord in Appendix 8. Hierbij werd gekeken naar het effect van captopril op de coronaire doorbloeding in het geisoleerde rattehart, alleen en in combinatie met isosorbide dinitraat. Hierbij bleek dat de vaatverwijdende eigenschappen van beide middelen afzonderlijk elkaar potentieerden. Dit effect was eveneens aanwezig bij de combinatie van isosorbide dinitraat met een andere sulfhydryl bevattende stof, cysteine, maar niet met ramiprilaat.

Op grond hiervan werd geconcludeerd tot een interactie met isosorbide dinitraat met mogelijk belangrijke consequenties voor de praktijk. Dit werd onderzocht in de laatste studie, Appendix 9. Hierbij kregen patienten met stabiele angina pectoris, die behandeld werden met een onderhoudsbehandeling isosorbide dinitraat, gerandomiseerd, dubbelblind, cross-over een enkelvoudige gift captopril of placebo. Toediening van captopril resulteerde in een significante verbetering bij inspanningsonderzoek. Tijdens inspanning werden geen significante verschillen ten opzichte van placebo gevonden wat betreft bloeddruk en hartfrequentie. Dit suggereert een sulfhydryl-afhankelijke interactie met de nitraatbehandeling, maar meer onderzoek is noodzakelijk, met name in vergelijking met ACE remmers zonder sulfhydryl groep.

De door ons uitgevoerde onderzoekingen staan niet op zichzelf, maar worden ondersteund en aangevuld door een groot aantal studies in de literatuur. Dit is samengevat in de Hoofdstukken II t/m IV. Hierbij komen in Hoofdstuk II respectievelijk het belang van het cardiale renine-angiotensine systeem, de potentiëring van het kinine-kallikreine systeem, de interacties met het prostaglandine metabolisme en het centrale zenuwstelsel, en ten slotte het belang van de sulfhydrylgroep uitgebreid aan de orde. Duidelijk is dat niet èèn bepaald mechanisme verantwoordelijk is voor het effect van captopril, maar juist een samenspel van een aantal verschillende werkingsmechanismen. Afhankelijk van het toepassingsgebied zal de nadruk meer op èèn afzonderlijk mechanisme komen te liggen. Het dierexperimenteel onderzoek maakt duidelijk dat captopril en andere ACE remmers differentiële effecten op hartfunctie, op coronaire doorbloeding, op myocardbeschadiging tijdens ischaemie/reperfusie, en op ventriculaire ritmestoornissen kunnen uitoefenen (Hoofdstuk III). De resultaten van andere onderzoekingen met ACE remmers in het geisoleerde

rattehart bevestigen onze gegevens. Het klinisch onderzoek (Hoofdstuk IV) laat zien dat er verschuiving plaatsvindt van de toepassing van captopril als laatste redmiddel bij hartfalen en hypertensie naar de vroege behandeling om verdere schade te voorkòmen. De behandeling bij angina pectoris, al of niet in combinatie met hypertensie of hartfalen, blijft vooralsnog experimenteel. Late interventie na het myocardinfarct, vanaf twee tot drie weken na het ontstaan, is reeds met succes toegepast. De klinische resultaten ten aanzien van de preventie en reductie van ernstige ventriculaire ritmestoornissen zijn beperkt en een eventuele bijdrage hiervan tot verlenging van de levensduur onbekend.

Dit proefschrift bewijst dat angiotensine converting enzym remmers meer zijn dan alleen perifere vaatverwijders. Wel degelijk bestaan er directe effecten op het hart zelf, waarbij meerdere mechanismen een rol spelen. Zoals uit het dierexperimentele onderzoek blijkt, kan dit neerkomen op cardioprotectieve eigenschappen tijdens ischaemie en reperfusie. Dit kan consequenties hebben voor de kliniek, al moet niet vergeten worden dat captopril in onze dierexperimenten soms in hoge concentraties gegeven werd.

Captopril lijkt echter zeker therapeutische mogelijkheden te hebben bij het voorkòmen van cardiale complicaties na het acute myocardinfarct, met name wanneer reperfusie spontaan of door middel van thrombolyse is opgetreden. Dit kan zich uiten in afname van de grootte van het myocardinfarct, verbetering van de hartfunctie, en vermindering van ritmestoornissen. Met een dubbelblind, placebo- gecontroleerd onderzoek, bedoeld om dit verder na te gaan, is reeds gestart. Vergelijkend onderzoek tussen captopril en andere ACE remmers zal duidelijk moeten maken of captopril een aparte positie inneemt door de aanwezigheid van een sulfhydryl groep. Dit geldt zeker ook voor de behandeling van angina pectoris, met name in combinatie met nitraat behandeling. In de komende jaren zal de rol van captopril en andere ACE remmers bij de behandeling van ischaemische hartziekte in het algemeen, en van het myocardinfarct in het bijzonder, ongetwijfeld in het middelpunt van de belangstelling komen te staan!

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